ence Library

MAR 11 1949

Volume 39 *Number 3*

Phytopathology

AN INTERNATIONAL JOURNAL

March 1949

Official Organ of

The American Phytopathological Society

$7.75 per year, Canada $7.50 per year, United States and dependencies $8.00 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph 4, Sec. 538, P. L. and R. Authorized May 12, 1921.

TABLE OF CONTENTS

Spraying experiments to control citrus canker ..... Liang Hwang 177

The conversion of strains of Alcaligenes radiobacter and Phytomonas tumefaciens in the "S" phase to the "M" phase of the heterologous species ..... Madeline F. Coleman and James J. Reid 182

Papaya virus diseases with special reference to papaya ringspot. D. D. Jensen 191

Papaya ringspot virus and its insect vector relationships. D. D. Jensen 212

Some relations between Phaseolus virus 2 and its associated crystalline inclusions ..... Saul Rich 221

The virus of tobacco leaf-curl ..... D. G. Sharp and F. A. Wolf 225

Virus concentration in plants acquiring tolerance to tobacco streak. Robert W. Fulton 231

SPRAYING EXPERIMENTS TO CONTROL CITRUS CANKER[1]

LIANG HWANG

(Accepted for publication October 1, 1948)

Citrus canker, caused by *Pseudomonas citri* Hasse, once came into prominence in Florida for a short time, but was eradicated from all commercial orchards there and in the other Gulf States of the United States. Fawcett (1) has recorded the known distribution of the disease in the Orient and other countries of the world, and Tanaka (6) has discussed the history of the canker in Japan, but the writer wishes to report briefly about the disease in South China. According to Lin (4), citrus canker was rather prevalent and destructive to sweet orange (*Citrus sinensis*) and mandarin orange (*C. nobilis*) in Foochow and Changchow of Fukien Province, but it was very slight on sweet orange in Gong Hsien and rather severe on lemon (*C. limonia*) in Nan-feng of Kiangsi Province. The writer (2) has reported that the disease is widely distributed in Kwangsi Province: it has been most destructive and prevalent on the pummelo group (*C. grandis*) in 8 of the 22 districts. Sweet orange and mandarin orange groups are less severely infected, while the tangerine group (*C. nobilis* var. *deliciosa*) and lemon are only slightly infected.

Since this disease is well established in most citrus growing areas in China, neither exclusion nor eradication are possible. An effective control measure is very necessary.

EXPERIMENTAL METHODS

A 4–6–50 Bordeaux mixture, alone or with materials to control insect pests, and a 1–500 lime solution, alone or with a sticker, were tested for their fungicidal efficacy in citrus orchards at the Kwangsi Agricultural Experiment Station, Sha-tang, Liu-chow, Kwangsi.

The Bordeaux mixture was used alone in 1940. In 1941, in order to control chewing insects, 0.5 lb. lead arsenate was added to each 50 gal. Bordeaux. The next two years, 1942 and 1943, a modified Bordeaux-oil emulsion was used so that wooly aphis and scale insects as well as the citrus canker bacteria might be controlled. The oil emulsion was prepared from 2 gal. vegetable oil (tea oil preferred), 2 lb. pure soap, and 1 gal. water. The mixture was boiled a few minutes and kept as a stock supply. Three parts by volume were added to 200 parts of Bordeaux mixture and thoroughly mixed at spraying time.

The lime solution was used alone in 1940. A sticker, 3 lb. alum to 50 gal. spray solution, was added in 1941. The lime solution sprays were somewhat unsatisfactory and were not used in the next two years.

[1] Thanks are due Messrs. W. N. Siang, I. Ching, C. M. Cheo, and others of the Division of Phytopathology and Entomology of Kwangsi Agricultural Experiment Station for help during the course of the experiments.

Three spray schedules were followed each year (Table 1). Schedule I was arranged so that sprays were applied when new flushes of foliage were produced by the trees. Schedules II and III were arranged so that spraying was done when temperatures were suitable for infection by the canker bacteria, but there were longer intervals between spray applications in Schedule III than in Schedule II.

Newly unfolded young leaves of citrus are most susceptible to the canker bacteria and protection of new foliage flushes is of prime importance in controlling the disease. According to S. L. Hsiong, new flushes appear about five times a year at Liu-chow: early February, late March, mid-May, mid-July, and September. New flushes depend upon frequency and abundance of precipitation and vary somewhat each year.

TABLE 1.—*Spray schedules for the control of citrus canker in Kwangsi Province, China, for 1940 through 1943*

Year	Schedule	Spraying dates
1940	I	June 15, July 16, Aug. 16
	II	June 6, Aug. 7, Aug. 31
	III	Aug. 7, Aug. 16, Aug. 31
1941	I	Apr. 17, July 17, Oct. 17
	II	Apr. 30, June 16, July 31
	III	May 3, July 1, Sept. 1
1942	I	Apr. 5, July 2, Oct. 1
	II	Apr. 16, May 28, July 16
	III	May 2, July 2, Sept. 7
1943	I	Mar. 17, May 30, July 30, Sept. 30
	II	Apr. 1, May 17, July 3, Aug. 17, Sept. 30
	III	Apr. 1, June 3, Aug. 2, Oct. 2

Young citrus leaves may be infected by the bacteria when temperatures range from 20° to 35° C. and free moisture is on the leaf surfaces for 20 minutes or longer (5). According to K. C. Chu's "Weather of China," atmospheric temperatures in the important citrus growing districts of Kwangsi Province were 19°–20.6° C. in April and 20.5°–23.7° C. in October in 1935. Upon checking weather records for 12 districts of the Province, the writer found that average temperatures during the 3-year period 1938–1940 were 19°–22.3° C. in April and 15.7°–20.2° C. in November. The precipitation was greatest between May and August and was least in December, January, and February.

In 1940 three orchards of 5-year-old trees were sprayed. They were *Citrus nobilis* var. *Poonensis* Hayada, *C. nobilis* var. *Unshiu* Swingle, and the very susceptible *C. grandis* Osbeck var. *Sha-tien*. In 1941 the same three varieties of citrus were sprayed, but a fourth variety, *C. sinensis* var. *Heung-shue-cheng* was included. Only trees of the very susceptible pummello group, *C. grandis* var. *Sha-tien*, were sprayed in 1942 and 1943. In the last year, 1943, large numbers of 2-year-old seedlings were sprayed: more than 100 seedlings of sour orange (*C. aurantium* var. *Kiang-tsin*), about the same number of Sunki seedlings (*C. nobilis* var. *Sunki*), and as many Mou-yu seedlings (*C. grandis* var. *Mou-yu*).

All trees were naturally infected with the citrus canker bacteria. Disease notes were recorded before spraying began and again two months after the final spraying in each schedule. Four disease classes were set up and plus and minus signs were used to designate variability within a class: Trace indicated an average of only one lesion for each ten leaves, Light indicated an average of only one lesion for each five leaves, Moderate signified an average of two lesions on each leaf and a few lesions on twigs and fruits, and Severe signified an average of five lesions on each leaf and numerous lesions on twigs and fruits.

RELATIVE EFFICACY OF BORDEAUX MIXTURE AND LIME SOLUTION

There was considerable natural infection in the orchards in 1940, and Bordeaux mixture was somewhat more satisfactory than lime solution in reducing the amount of bacterial canker infection (Table 2). In spite of the fact that Lee (3) suggested that 1–500 lime solution was the cheapest and most effective spray in orchard practice, the results reported here indicate that Bordeaux mixture is to be preferred to lime solution. In 1941 there was relatively little natural infection in the orchards and the advantages of spraying to control citrus canker bacteria were not evident.

TABLE 2.—*The effect of Bordeaux mixture (4–6–50) and lime solution (1–500) on citrus canker in three varieties of citrus in 1940*

Species and variety of citrus	Fungicide	Spray schedule[a]	Infection on trees	
			Before spraying	After spraying
C. nobilis Poonensis	Bordeaux	I	Moderate –	Trace –
		II	Moderate +	Trace –
		III	Moderate	None
	Lime solution	I	Moderate	None
		II	Moderate –	None
		III	Severe	Trace –
C. nobilis Unshiu	Bordeaux	I	Trace +	None
		II	Trace	None
		III	Light +	None
	Lime solution	I	Trace +	Light –
		II	Trace +	Light +
		III	Light +	Trace
C. grandis Sha-tien	Bordeaux	I	Moderate +	None
		II	Moderate +	None
		III	Moderate +	None
	Lime solution	I	Moderate +	Trace –
		II	Moderate –	Trace –
		III	Moderate	Trace

[a] Dates for each spray schedule are in table 1.

In the absence of insects in the orchards, Bordeaux mixture alone was satisfactory; when chewing insects were present the addition of lead arsenate insecticide proved to be good orchard practice and was not injurious to citrus foliage.

TABLE 3.—*The effect of Bordeaux-oil emulsion on citrus canker in Citrus grandis var. Sha-tien in 1942 and 1943*

Spray schedule	Infection[a] on trees in			
	1942		1943	
	Before spray	After spray	Before spray	After spray
I	Light	Trace	Trace	None
II	Light +	Light +	Trace +	None
III	Light −	Trace −	Light −	None
Check	Light −	Light	Trace	Light

[a] Results are averages for 8 trees in each schedule and in check plots.

THE EFFICACY OF BORDEAUX-OIL EMULSION

This spray, applied to trees of the very susceptible *Citrus grandis* var. *Sha-tien*, reduced bacterial infection when applied according to Schedules I and III in 1942 and when applied according to all three schedules in 1943. There was no reduction of infection when it was applied according to Schedule II in 1942 (Table 3). It is not known why Schedule II was not effective in 1942. It may have been because the final spray was applied on July 16 whereas the final spray in Schedule I was made on October 1 and that of Schedule III was made on September 7 in that year.

Seedling stocks were more variable with respect to the infection they carried after having been sprayed with the Bordeaux-oil emulsion. Since rather large numbers of seedlings were in each plot, the percentages of seedlings in each disease class were recorded (Table 4). The spray effectively reduced infection in the sour orange seedlings, but failed to do so in the Sunki and Mou-yu seedlings.

TABLE 4.—*The effect of Bordeaux-oil emulsion spray on citrus canker in seedling stocks in 1943*

Seedlings of citrus		Spray schedule	Infection before spraying	Percentage of trees in each disease class after spraying				
Kind	Number			None	Trace	Light	Moderate	Dead[a]
Sour orange	46	I	Tr to Lt	61	26	0	0	13
	31	II	do	87	10	0	0	3
	31	III	0 to Lt	78	23	0	0	0
	30	Check	Tr	36	53	3	0	7
Sunki	47	I	Tr	0	34	58	0	9
	32	II	Tr	0	50	34	3	13
	32	III	Tr	0	50	41	6	3
	32	Check	Tr	0	56	34	9	0
Mou-yu	36	I	Tr	0	67	25	0	8
	36	II	0 to Tr	0	64	25	6	6
	36	III	Tr	0	75	17	0	9
	36	Check	Tr	0	50	19	6	25

[a] Death was due to causes other than bacterial canker.

CONCLUSIONS AND SUMMARY

In three of four years the incidence of citrus canker in orchards of 5-year-old trees in Kwangsi Province was reduced by spraying with Bordeaux mixture or with Bordeaux-oil emulsion. Spray schedules set up according to the appearance of new foliage flushes or according to the temperatures favorable for the canker bacteria were satisfactory if they began early in the growing season and extended to late September or early October. Thorough spraying during the growing season not only protected citrus foliage from infection but greatly reduced the amount of infection that had been established on the trees prior to the first spray.

Because wooly aphis and scale insects are prevalent in citrus orchards of Kwangsi Province, the Bordeaux-oil emulsion probably will be the most satisfactory and economical spray material for general orchard practice.

In a single season the Bordeaux-oil emulsion effectively controlled citrus canker in sour orange seedling stocks, but it failed to control the disease in stocks of the very susceptible Sunki and Mou-yu seedlings. At present it is not possible to recommend sprays for nursery practice.

DEPARTMENT OF PLANT PRODUCTION,
COLLEGE OF AGRICULTURE,
NATIONAL KWANGSI UNIVERSITY,
KWEI-LIN, CHINA

LITERATURE CITED

1. FAWCETT, H. S. Citrus diseases and their control. Ed. 2. 656 pp. McGraw-Hill Book Co., New York. 1936.
2. HWANG, LIANG. [Citrus diseases in Kwangsi]. In Chinese. Reprinted from Kwangsi Agriculture 4(1): 27–61. 1943.
3. LEE, H. A. Action of some fungicides on the citrus canker organism. Philippine Jour. Sci. 17: 325–341. 1920.
4. LIN, K. S. [Citrus disease survey in Fukien and Kiangsi]. In Chinese. Lingnan University, Col. Agr., Res. Bul. 7. 1944.
5. PELTIER, G. L. Influence of temperature and humidity on the growth of Pseudomonas citri and its host plants and on the infection and development of the disease. Jour. Agr. Res. [U.S.] 20: 447–506. 1920.
6. TANAKA, T. A brief history of the discovery of citrus canker in Japan and experiments in its control. Fla. State Plant Bd. Quart. Bul. 3: 1–15. 1918.

THE CONVERSION OF STRAINS OF ALCALIGENES RADIOBACTER AND PHYTOMONAS TUMEFACIENS IN THE "S" PHASE TO THE "M" PHASE OF THE HETEROLOGOUS SPECIES

MADELINE F. COLEMAN AND JAMES J. REID

(Accepted for publication October 28, 1948)

The cultural and morphological similarity of *Phytomonas tumefaciens* and *Alcaligenes radiobacter* has been recognized for many years. Patel (8) noted that the two organisms were similar in their morphological and physiological characteristics and could be separated only by pathogenicity. Similarity between certain non-pathogenic organisms and the crown-gall producing organism has been reported by Sagen, Wright, and Riker (11). Morphological and physiological studies continued by Riker et al (9) were conducted in an attempt to obtain cultural differences by growth in various media. Serological studies showed no cross agglutination between species under the conditions of their experiments. No serological relationship in the Dawson "M" phase[1] was observed and reported by us (1) in cross agglutination tests. Sagen, Riker, and Baldwin (10) reported a further study of the physiological characteristics of *Phytomonas tumefaciens*, *Phytomonas rhizogenes*, and *Alcaligenes radiobacter* with emphasis on their nitrogen and carbon metabolism. No separation was effected by this study. The attempts of Hendrickson, Baldwin, and Riker (7) to induce variation by repeated transfer on different artificial media met with failure. Variations in the physiological behavior of the crown-gall organism were obtained by plant passage. The authors were unable to generalize on physiological characteristics but found that virulent cultures of crown-gall bacteria secured in plant passage experiments gave *radiobacter* reaction on laboratory media. This would seem to indicate that, by laboratory procedures and plant passage, cultures which were doubtless originally crown-gall organisms had lost their pathogenicity and thus resembled *A. radiobacter* or had acquired some of the physiological characteristics of *A. radiobacter* without losing their pathogenicity. The authors suggested that such changes might occur in which the non-pathogenic crown-gall could be induced to assume the physiological characters of *A. radiobacter* or that if pathogenic crown-gall cultures which are physiologically similar to *A. radiobacter* were to lose their pathogenicity, *Phytomonas tumefaciens* might be converted to resemble *A. radiobacter*. They believe that in view of the results obtained in their study it is possible to bring about a transference of *Phytomonas tumefaciens* into an organism possessing the important characters of the "so-called" *Alcaligenes radiobacter*.

In general, the results of these extensive studies and others not cited

[1] Designation used here follows terminology suggested in (4).

here serve to emphasize the close morphological and physiological relationship between the crown-gall and *radiobacter* organisms. In an endeavor to discover the reasons for their close similarity, it seemed feasible to investigate the serological relationship of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* along methods described by Dawson (2, 3) and Dawson and Sia (5) in which their serological techniques in the transformation of pneumococcal types could be applied. In a previous paper (1) the results of an investigation of the serological relationship of *A. radiobacter* and *P. tumefaciens* with the use of agglutination and agglutinin absorption techniques have been reported. This present paper deals with a continuation of that study in which the conversion of the same strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* in the Dawson "M" and "S" phases to the heterologous species is described.

METHODS

Cultures used. Cultures representing both the "M" and "S" phases of three strains of *Alcaligenes radiobacter*, Ri-Ia, R3, and R3scI, and the "M" and "S" phases of two strains of *Phytomonas tumefaciens*, ScT5fffI and ScA-I, were used in this study.[2] The history of these strains and the methods used in deriving the "S" phase variants have been discussed in a previous publication (1).

Media employed. Yeast extract-mannitol-mineral salts broth or agar, considered the standard media for organisms of this mucoid type, were used for cultivation in the "M" phase. For decapsulation studies and maintenance of the Dawson "S" phase cells, the mannitol content was reduced to 0.5 per cent, in the presence of 10 per cent homologous "M" antiserum. The omission of mannitol in the *in vitro* experiments in encapsulating "S" phase cells to the "M" phase of the heterologous species produced quicker results.

Serological techniques. Methods used in animal immunization and agglutination and agglutinin absorption techniques employed have been described (1). Macroscopic agglutination tests were run routinely to check "M" and "S" phases of the cultures whenever used throughout this study.

In Vitro Experiments

The method used to encapsulate *Phytomonas tumefaciens* in the "S" phase with the *Alcaligenes radiobacter* "M" phase capsule and to place the *Phytomonas tumefaciens* "M" phase capsule on the "S" phase *Alcaligenes radiobacter* was essentially the one described by Dawson (2, 3) and Dawson and Sia (5) for the conversion of the pneumococci.

[2] The cultures used in this study were obtained from the stock culture collection of the Division of Bacteriology, New York State Agricultural Experiment Station, Geneva, N. Y., through the courtesy of Dr. A. W. Hofer. These strains have been employed by a number of research workers in the fields of soil bacteriology and plant pathology. The strains of *P. tumefaciens* used were ScT5fffI and ScA-I. These are single-cell isolates made at the University of Wisconsin, known as typical of their species. *A. radiobacter* R3 was isolated by D. B. Fred of the University of Wisconsin in 1927. R3scI is a single-cell isolation from culture R3.

Capsular material was prepared by growing the "M" phases of the strains under investigation on standard yeast-mannitol-mineral salts agar in large Blake bottles. After 72 hours, the cultures were Gram stained and removed by washing with sterile saline and then filtered aseptically through sterile cotton into sterile vaccine bottles. Enough of each suspension was removed to use for checking its Dawson "M" phase by macroscopic agglutination tests against "M" and "S" phase antisera. The remainder was then heated in a water bath for 30 minutes at 60° C., with gentle agitation. The heat-killed vaccines were kept at room temperature for one week and tested daily for sterility by cultivation in yeast-mannitol-mineral salts broth and by streaking on plates of the same medium. The vaccines were then stored at 4° C. and sterility tests made whenever used again.

Encapsulation of the "S" phases of the strains under investigation was next attempted. Several alternate conditions were set up in the *in vitro* experiments which varied in the following manner:

1. Difference in size of inoculum of the organism to be encapsulated.
2. Difference in amount of the desired heterologous "M" phase, heat-killed vaccine.
3. Difference in amount and type of antisera.
4. Difference in mannitol content of standard yeast-mannitol-mineral salts broth or agar.

The procedure which proved successful consisted of transferring one drop of a 10^{-6} dilution of an actively growing culture in the "S" phase to 1 ml. of yeast extract-mineral salts broth to which was added 1.0 ml. of heat-killed vaccine of the desired "M" phase of the heterologous organism and 0.1 ml. of antisera prepared against the "S" phase of the homologous strain. This was done in triplicate for each strain.

These cultures were kept at room temperature and transfers were made every 24, 48, and 72 hours, by streaking and making pin point colonies on yeast-mannitol-mineral salts agar plates. Observations of these plates were made every 24, 48, and 72 hours for typical mucoid colonies, and any suspected "M" phase colony was transferred to yeast-mannitol-mineral salts agar slants. After Gram staining, antigenic suspensions were made from 48-hour cultures to test against the heterologous and homologous "M" and "S" phase antisera. Antisera controls were run at the same time.

In Vivo Conversion

Plant Inoculations. According to the methods described by Smith and Townsend (12) and Hendrickson et al (7), individually potted six-week-old tomato plants, kept under optimal conditions in the greenhouse, were employed for the *in vivo* tests. Plants were segregated into isolated groups as follows and inoculated with 48-hour, actively growing cultures of the several strains under study:

1. One group with "M" phase *Phytomonas tumefaciens.*
2. One group with "S" phase *Phytomonas tumefaciens.*
3. One group with "M" phase *Alcaligenes radiobacter.*
4. One group with "S" phase *Alcaligenes radiobacter.*
5. One group with an "S" phase *Phytomonas tumefaciens* culture converted *in vitro* to "M" phase *Alcaligenes radiobacter* (Conversion described later in this paper).

Plant inoculations were made by taking up a little of the culture on a sterile straight needle and puncturing the plant at the internodes (12), averaging three inoculations on one tomato plant. Three plants were used in each group for each isolate under investigation. Sterile needle punctures were likewise made on control plants. Segregation of these various groups was carefully observed and plants were watered by placing water in the bottom of the troughs in which they stood. They were observed daily for any change or appearance of tumorous growth.

Plant Isolations. After six weeks, isolations were made by removing a part of the excess growth adjacent to the plant stem with a sterile razor blade and disinfecting the surface of this plant material by passing it through 50 per cent alcohol and rinsing in sterile distilled water. After this plant material was made homogenous by crushing with a sterile pestle, a drop of this suspension was transferred to yeast-mannitol-mineral salts broth and allowed to grow for 24 hours before streaking on yeast-mannitol-mineral salts agar plates. In the case of crown-gall forming isolates, aniline blue was added to the standard medium to facilitate separation (11). After 48 hours, isolated and typical mucoid colonies, checked first by the Gram stain, were transferred to yeast-mannitol-mineral salt agar slants. Subculturing was carried out by repeating the above procedure until pure cultures were obtained. When the purity of each isolation was certified, antigenic suspensions were prepared and agglutination tests were made with all the antisera on hand.

RESULTS

In Vitro Experiments. The *in vitro* experiments to convert *Phytomonas tumefaciens* "S" phase cells to *Alcaligenes radiobacter* "M" phase cells were successful under two conditions. The first culture was obtained when one drop of a 10^{-6} dilution of the "S" phase culture was grown in 1 ml. of yeast-mannitol (containing half the amount of mannitol)-mineral salts broth together with 0.5 ml. of heat-killed "M" vaccine of the heterologous organism with 0.2 ml. of the homologous "S" phase antiserum. Succeeding cultures were obtained more rapidly when the strains were grown in mannitol-free, yeast-mineral salts broth and 1.0 ml. of heterologous "M" phase heat-killed vaccine plus 0.1 ml. of the homologous "S" antiserum. It may be possible that the absence of mannitol induced the organism to utilize the carbohydrate in the vaccine material. "M" phase colonies which appeared mucoid were obtained from 48-hour-

old broth cultures on yeast-mannitol-mineral salts agar plates. Gram stains attested the purity of these new isolations and transfers to agar slants of the same medium were made to obtain cultures to check by agglutination tests, the results of which are shown in table 1.

Agglutination occurred only with the *Alcaligenes radiobacter* "M" phase antisera which would seem to indicate the conversion of the *Phytomonas tumefaciens* "S" phase strains to a serological type related to the "M" phase *Alcaligenes radiobacter* strains.

Similar techniques in endeavors to place the *Phytomonas tumefaciens* capsule on the *Alcaligenes radiobacter* "S" phase strains were not successful. When grown in *Phytomonas tumefaciens* "M" phase heat-killed vaccine and *Alcaligenes radiobacter* "S" antiserum, it was found to revert

TABLE 1.—*Results of the agglutination tests of "S" phase Phytomonas tumefaciens strains converted in vitro to Alcaligenes radiobacter "M" phase serological type*

Antisera	Agglutination[a] in dilutions of "S" phase *Phytomonas tumefaciens* antigens									
	40	80	160	320	640	1280	2560	5120	10240	Control
Alcaligenes radiobacter "M"	2+	2+	2+	2+	3+	2+	2+	2+	+	–
Alcaligenes radiobacter "S"	±	±	±	±	–	–	–	–	–	–
Phytomonas tumefaciens "M"	–	–	–	–	–	–	–	–	–	–
Phytomonas tumefaciens "S"	±	±	±	±	–	–	–	–	–	–

[a] Agglutination: – = none, ± = doubtful, 1 = slight; 2 = fair; 3 = good.

to its homologous "M" phase. When grown in *Phytomonas tumefaciens* capsular material in the presence of both the *Alcaligenes radiobacter* "M" and "S" phase antisera, it retained the homologous "S" phase. These results were obtained consistently and indicated by agglutination tests.

In Vivo Experiments. The results of the *in vivo* experiments are in table 2. *Phytomonas tumefaciens* strains in the "M" phase induced a very typical growth in 15 days. A 24-hour-old *Phytomonas tumefaciens* "S" phase culture which showed serological relationship to *Alcaligenes radiobacter* "M" phase culture (Table 1) produced small and abortive tumors, apparent 17 days after inoculation. These tumors became dry and amorphous and ceased developing in a few days with no effect on the growth of the plants. Plant inoculations made after this culture had been carried on laboratory media for 18 days, after the first culture had been obtained, failed to produce tumors. Following inoculations with "S" phase cells of *Phytomonas tumefaciens* tumors formed slowly and were not vigorous.

Plant inoculation with *Alcaligenes radiobacter* "M" phase cells did not result in any tumor formation. "S" phase cells, however, when rubbed

into an aseptically bruised part of the tomato plant stem produced the first evidence of abnormal plant cell growth 3 and 7 days after inoculation. In repeated experiments with all the strains of *Alcaligenes radiobacter* "S" phase cells, a good tumorous growth occurred and resulted in a marked stunting of plant growth. The same was observed with plants inoculated with "M" phase cells of *Phytomonas tumefaciens* strains.

Plant Isolation Results. To determine the validity of tumor production, isolations from the experimental plant tumors were made so that antigenic suspensions could be prepared to use for serological identification

TABLE 2.—*Results obtained in 1944 of plant inoculation studies in tomato plants*

Date of inoculation	Inocula	Tumor growth and date of first appearance	Date for well formed tumors	Date of isolation
March 27	*Alcaligenes radiobacter* "M" cells	none	.....	.....
March 27	*Phytomonas tumefaciens* "S" cells with *A. radiobacter* capsule[a]	April 13	April 20	May 11
March 27	*P. tumefaciens* "M" cells	April 11	April 13	May 11
April 11	*P. tumefaciens* "S" cells with *A. radiobacter* capsule[b]	none	.....	.....
April 11	*A. radiobacter* "S" cells[c]	April 14	April 20	May 11
April 13	*P. tumefaciens* "S" cells	May 10	May 16	May 27

[a] First culture obtained in the laboratory which showed serological relationship to the *Alcaligenes radiobacter* "M" phase strains.

[b] Later cultures which had been carried in the laboratory for sufficient length of time to permit stabilization of the new "M" phase.

[c] Another inoculum which produced tumors within 7 days is not indicated.

(Table 3). Plant passage did not modify the serological properties of *Phytomonas tumefaciens* in the "M" phase, because agglutination occurred only with its homologous "M" phase antisera. In the "S" phase, it was agglutinated only by its homologous "S" and heterologous "S" phase antisera, thereby indicating antigenic similarity to *Alcaligenes radiobacter* "S" phase cells and substantiating the studies reported previously (1). The culture obtained from the tumors produced by inoculating *Alcaligenes radiobacter* "S" phase cells into an aseptically bruised plant internode showed no agglutination by its homologous "S" or "M" phase antisera. On the other hand, this isolate was agglutinated only by the *Phytomonas tumefaciens* "M" phase antisera. This would seem to indi-

TABLE 3.—*Serological Tests of Isolates from Experimental Tumors*

Antisera	Inocula and plant isolates	Agglutination[a] at dilutions of								
		40	160	320	640	1280	2560	5120	10240	Control
Phytomonas tumefaciens "M"	*Phytomonas tumefaciens* "M"	3	3	3	3	4	4	4	2[a]	–
Do	*Alcaligenes radiobacter* "S"	2	2	2	2	2	2	2[b]	–	–
Do	*Phytomonas tumefaciens* "S"	1	1	1	–	–	–	–	–	–
Do "S"	Do	2	3	3	3	3	3	2	–	–
Alcaligenes radiobacter "M"	*Phytomonas tumefaciens* "M"	–	–	–	–	–	–	–	–	–
Do	Do "S"	–	–	–	–	–	–	–	–	–
Do	*Alcaligenes radiobacter* "S"	–	–	–	–	–	–	–	–	–
Do "S"	Do	–	–	–	–	–	–	–	–	–
Do	*Phytomonas tumefaciens* "S"	1	1	1	1	1	1	1	–	–

[a] Agglutination: – = none; 1 = slight; 2 = fair; 3 = good; 4 = excellent.

[b] Shows serological relationship to *Phytomonas tumefaciens* "M." Agglutinated by heterologous "M" antisera and not by homologous "M" or "S" phase antisera.

cate the *in vivo* conversion of *Alcaligenes radiobacter* "S" phase to the *Phytomonas tumefaciens* "M" phase.

In the case of *Alcaligenes radiobacter* "M" phase inoculum and the *in vitro* converted *Phytomonas tumefaciens* strains, no contributable material for serological tests was obtained since no tumors were produced.

DISCUSSION OF RESULTS

The results of this study seem to indicate that the organisms recognized as *Alcaligenes radiobacter* and *Phytomonas tumefaciens* comprise a single species and in their usual "M" phases bear the same relation to each other as do the types of the pneumococcus (2, 6) to one another. The failure, under conditions of the experiments, in placing the *Phytomonas tumefaciens* capsule on the *Alcaligenes radiobacter* "S" phase cell, but the success of the *in vitro* attempts with the "S" phase of *Phytomonas tumefaciens* suggests that the latter represents a relatively unstable form. It would appear that the "M" phase recognized as *Alcaligenes radiobacter* is the normal and stable phase in nature and that the "M" phase recognized as *Phytomonas tumefaciens* represents a transitory adaptation. Differences in the relative stability of the strains of these organisms were significant throughout these investigations. *Alcaligenes radiobacter* stimulated better antibody production than did *Phytomonas tumefaciens* (1). In decapsulation studies, *Phytomonas* strains proved less resistant to the presence of the homologous antisera and lost the capsular substance in the course of 7 serial transfers (1). *Alcaligenes* strains, on the other hand, were resistant to homologous antiserum of higher titer and as many as 19 transfers were required to remove the capsule (1). Moreover, in capsule conversion attempts, the "S" phase of *Phytomonas* strains slowly yielded and acquired the capsule of *Alcaligenes radiobacter*, while in the *in vitro* attempts to convert the "S" phase of *Alcaligenes radiobacter* to the "M" phase of *Phytomonas tumefaciens* that was not accomplished, but conversion was only brought about *in vivo*.

It is interesting to note that Hendrickson et al (7) suggested that in view of the results obtained in their study it is possible to bring about a transference of *Phytomonas tumefaciens* into an organism possessing the important characters of the "so-called" *Alcaligenes radiobacter*. The results of our studies would seem to indicate that this was accomplished and may offer an explanation for the baffling similarities which have persistently resisted the efforts of notable workers in this field (7, 9, 10, 11) in attempts to differentiate *Phytomonas tumefaciens* and *Alcaligenes radiobacter* by morphological and physiological characteristics.

SUMMARY

For the strains under investigation in this study, conversion *in vitro* of *Phytomonas tumefaciens* to *Alcaligenes radiobacter* was brought about by

placing the *Alcaligenes radiobacter* capsule on the *Phytomonas tumefaciens* "S" phase cell.

Although difficulty was encountered in attempts to effect the conversion of *Alcaligenes radiobacter* to *Phytomonas tumefaciens in vitro*, this conversion was effected *in vivo* by inoculating suitable host plants with the *Alcaligenes radiobacter* strains in the "S" phase.

The conversion of *Phytomonas tumefaciens* to a form serologically identical with *Alcaligenes radiobacter* in the "M" phase was accompanied by loss of the ability to attack a susceptible host plant.

The removal of the *Alcaligenes radiobacter* "M" capsule changed this organism from a state of avirulence to one of significant virulence which in the presence of a suitable host induced the formation of a capsule of the *Phytomonas tumefaciens* serological type.

THE DEPARTMENT OF BACTERIOLOGY
THE PENNSYLVANIA STATE COLLEGE
STATE COLLEGE, PA.

LITERATURE CITED

1. COLEMAN, M. F., and J. J. REID. A serological study of strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* in the "M" and "S" phases. Jour. Bact. 49: 187–192. 1945.
2. DAWSON, M. H. The transformation of pneumococcal types. I. The conversion of R forms of pneumococcus into S forms of the homologous type. Jour. Exp. Med. 51: 99–122. 1930.
3. ————. The transformation of pneumococcal types. II. The interconvertibility of type-specific S pneumococci. Jour. Exp. Med. 51: 123–147. 1930.
4. ————. Variation in the pneumococcus. Jour. Path. and Bact. 39: 323–344. 1934.
5. ————, and R. H. P. SIA. In vitro transformation of pneumococcal types. I. A technique for inducing transformation of pneumococcal types in vitro. Jour. Exp. Med. 54: 681–700. 1931.
6. GRIFFITH, F. The significance of pneumococcal types. Jour. Hyg. 27: 113–159. 1928.
7. HENDRICKSON, A. A., I. L. BALDWIN, and A. J. RIKER. Studies on certain physiological characters of *Phytomonas tumefaciens*, *Phytomonas rhizogenes*, and *Bacillus radiobacter*. Part II. Jour. Bact. 28: 597–618. 1934.
8. PATEL, M. K. Biological studies of Pseudomonas tumefaciens Sm. & Town., and fifteen related non-pathogenic organisms. Iowa State Coll. Jour. Sci. 3: 271–298. 1929.
9. RIKER, A. J., W. M. BANFIELD, W. H. WRIGHT, G. W. KEITT, and H. E. SAGEN. Studies on infectious hairy root of nursery apple trees. Jour. Agr. Res. [U.S.] 41: 507–540. 1930.
10. SAGEN, H. E., A. J. RIKER, and I. L. BALDWIN. Studies on certain physiological characters of *Phytomonas tumefaciens*, *Phytomonas rhizogenes*, and *Bacillus radiobacter*. Part I. Jour. Bact. 28: 571–597. 1934.
11. ————, W. H. WRIGHT, and A. J. RIKER. The cultural differentiation of *B. radiobacter* (Beij.) and closely related organisms. (Abstr.) Jour. Bact. 17: 22–23. 1929.
12. SMITH, E. F., and C. O. TOWNSEND. A plant-tumor of bacterial origin. Science 25: 671–673. 1907.

PAPAYA VIRUS DISEASES WITH SPECIAL REFERENCE TO PAPAYA RINGSPOT[1]

D. D. JENSEN[2]

(Accepted for publication November 1, 1948)

INTRODUCTION

Papaya (*Carica papaya* L.) is the third most important fruit crop grown in the Territory of Hawaii, being exceeded in commercial value only by pineapple and banana. In 1945 a previously undescribed papaya disease was encountered at Kailua on the island of Oahu. The discovery of the disease, named papaya ringspot, was published by Lindner *et al.* (32) in 1945. Experiments reported in abstract form by Jensen (24, 25) in 1946 and 1947 demonstrated the virus nature of the disease, its incubation period in the plant, and that *Myzus persicae* (Sulzer) and three unnamed *Aphis* species were capable of transmitting the virus. Failure to infect papaya with five common virus diseases occurring on Oahu or to transmit ringspot virus to plants other than papaya was also reported.

In 1947 Hendrix and Matsuura (19) reported juice transmission of ringspot virus to healthy papaya by the use of carborundum and also by hypodermic injection. The disease was not found to be transmitted through the seed.

Lindner *et al.* (33) in 1947 reported field observations on the rate of disease spread and on the effect of adverse environmental conditions on ringspot trees. Holmes *et al.* (22) in 1948 published results of a ringspot disease survey made on the main islands of the Hawaiian group. The disease was found only on Oahu. The report also stated that 15 varieties, strains, and hybrids of papaya tested were susceptible to the disease. Possible control procedures were also discussed.

The present paper reports the results of experiments on papaya ringspot disease carried out on the island of Oahu during 1945 and 1946. Included is a discussion of the symptoms and incubation period of the disease in the foliage, stem, and fruit of the papaya, rate of virus movement through the plant, and host range investigations. The virus-vector relationships of papaya ringspot are discussed in a separate paper (26). The writer's departure from Hawaii, terminating the investigation, accounts for the preliminary nature of certain phases of the study, such as virus movement through the plant. Since most of the known facts regarding the occurrence and types of papaya virus diseases throughout the world have been reported only within the past 2 decades and because this information has never been summarized, a review of the reported viruses of

[1] Published with the approval of the Director as Technical Paper No. 174 of the University of Hawaii Agricultural Experiment Station.

[2] Formerly Assistant Entomologist, University of Hawaii Agricultural Experiment Station. Now Assistant Professor of Entomology and Assistant Entomologist, University of California, Berkeley, California.

papaya is included. The possible relationships of some of these viruses are also discussed.

LITERATURE REVIEW

The first report of a papaya disease being attributed to a virus was made by Smith (47) in 1929 in Jamaica. In later accounts (47) he stated that papaya mosaic was primarily responsible for the absence of large-scale papaya cultivation in Jamaica. Smith described the disease as causing yellowing and mottling of the leaves, accompanied by reduction of the terminal leaves to a pencil point. The larger leaves meanwhile hang down and the plant gradually dies. Buds are often put out lower down on the stem but they usually also become diseased and die. Later reports on the Jamaica virus were published by Edwards (16) in 1933, by Larter (30, 31) from 1935 to 1943, and by Martyn (35) in 1942. No transmission experiments have been reported from Jamaica although Larter tested 29 types of papaya for resistance to the disease in the field. In 1937 one of these, *Carica cauliflora* Jacquin from Panama, was reported to be immune to the disease.

A curly-leaf disease of papaya was reported from Santo Domingo in 1930 by Ciferri (13). Symptoms include curling and twisting of the younger leaves to form cushions, yellow and green mosaic spots on the older leaves, followed by yellowing and drying up of the leaves, both old and young, within two months. A succession of new growth develops which in turn becomes diseased and falls from the plant. These conditions last for several months to one or two years before the plant dies. The upper part of the main stem of diseased trees develops irregular, elongate suberous epidermal growths resulting from the formation of layers of cork under the epidermis.

Although Ciferri reported transmission of curly leaf disease to 29 of 78 healthy plants by inoculation with latex from infected plants, he was uncertain as to the cause of the disease and did not suggest a virus origin. His positive cases varied from one out of 18 in one experiment to 18 out of 20 in another. Since Ciferri did not report that his plants were protected from natural spread and no controls were mentioned, latex transmission of curly-leaf disease needs confirmation.

Three virus diseases, bunchy top, die-back, and mosaic, occur in Puerto Rico. Bunchy top, first reported by Cook (14) in 1931, has been the major limiting factor in papaya production on the island since 1937 (7). J. H. Jensen (27, 28) in 1938 and 1939 described the disease symptoms and transmission experiments. Infected plants become stunted, produce chlorotic leaves with rigid, short petioles which extend horizontally rather than nearly vertically from the main stem. In advanced stages the trees are denuded except for a few small leaves at the top. The petioles and stem develop dark green, elongate streaks. Diseased plants produce very little latex from wounds. A high percentage of diseased trees is killed.

Attempts to transmit the virus mechanically and by grafting were unsuccessful. J. H. Jensen found a new species of leafhopper breeding on papaya which was described by Oman (38) in 1937 as *Empoasca papayae*. Nymphs and adults of this insect were caged on young, healthy papaya plants for 10 days. Three months after the insects were removed, the test plants developed symptoms resembling those of bunchy top. Soon thereafter the plants died as a result of root-rot fungus. In the meantime Jensen left Puerto Rico and the experiment was not repeated until several years later. In 1946 and 1947 Adsuar and Sein (3, 43) reported experiments which confirm the identity of *Empoasca papayae* as the vector of papaya bunchy top virus.

Kevorkian (29) in 1938 reported that the lace bug, *Corythuca gossypii* L., and the stink bug, *Nezara viridula* L., failed to transmit bunchy top. Of particular interest is the observation that bunchy top plants appear to be more acceptable as host plants of the lace bug than are healthy plants. Colonies are usually found only on diseased plants, and the insect is present on most diseased plants. The disease usually precedes the infestation.

Papaya die-back, mentioned briefly by Adsuar (4) in 1946, is characterized by severe chlorosis, defoliation, and necrosis of the apex of the stem. The symptoms resemble those of bunchy top but are much more severe.

In 1946 Adsuar (4, 5, 6) described a papaya mosaic from Puerto Rico which is transmissible by mechanical inoculation, by grafting, and by means of the green citrus aphid, *Aphis spiraecola* Patch. Plants develop symptoms in from 8 to 21 days. Progressive symptoms include chlorosis, mottling, wrinkling, and puckering of the top leaves. As the disease progresses, the plant becomes stunted and the leaves are severely deformed and may be reduced to filiform structures. The stem develops dark green, elongate streaks and the fruits have green and dark brown rings.

In 1945 Goenaga (17) reported that a leaf-roll virus of tobacco had spread in Puerto Rico to nearby plants representing several plant families. Young papaya trees reportedly acquired the disease when placed in contact with diseased potato plants. The symptoms on papaya resemble those of bunchy top. Since Goenaga's observations with regard to papaya have not been confirmed experimentally, the identity of the papaya disease and the conclusion that it was a tobacco virus and that it had spread from potato must yet be proved.

Stell (48) in 1937 stated that no commercial cultivation of papaya in Trinidad had been attempted and that such an undertaking would probably fail. Papaya mosaic was listed as the first of two major diseases of this plant which would hinder successful cultivation. Baker (9) in 1939 described Trinidad mosaic symptoms and stated the disease was severe in all districts of the colony. Marked mosaic of the leaf lamina and water-soaked areas or oil spots on the petioles constitute the first symptoms of the disease. According to Baker's illustration, the leaf mosaic consists of

conspicuous chlorotic spots and irregular chlorotic areas spreading out from the veins and veinlets. No new terminal leaves are produced after the growing point becomes diseased. In about two months after symptoms appear the top of the tree dies and secondary rotting sets in which is arrested one or two feet below the apex. The tree may be killed outright in a short time. However, usually axillary shoots are produced, the lower of which sometimes remain healthy and bear fruit.

Trinidad mosaic has not been transmitted experimentally. Baker tested 14 species of homopterous insects with negative results. On the basis of symptoms and spread there can be little doubt that the disease is due to a virus despite Castelli's (12) contention in 1939 that the disorder is a result of improper soil conditions.

Acuna and Zayas (1, 2) in 1940 and 1946 described two diseases of papaya in Cuba apparently caused by viruses. One, called type A, is considered to be the same as bunchy top in Puerto Rico. Type B, known as Cotorro mosaic and limited to Havana, is more destructive than type A. The leaves develop chlorotic, translucent areas which extend out from the veins. New leaves become twisted and otherwise distorted. The interveinal tissue is puckered and reduced to the extent that the veins are crowded together and often run parallel. The bases of the young petioles and sometimes the stems develop green or greenish-red areas which are annular in form. As the plant grows, the color of the spot becomes normal in one zone, thus changing the form from a ring to a hook or a U with unequal arms. Spots sometimes also appear on the fruit. In some plants the basal leaves persist and the upper part of the stem is completely denuded or carries a new flush of leaves at the apex with the intervening space bare. In contrast to bunchy top, the latex flows freely from plants having Cotorro mosaic.

In South America, papaya diseases attributed to viruses have been reported from British Guiana, Brazil, and Venezuela.

In 1934 Martyn (34) reported a papaya disease from British Guiana, described only as curly leaf, which spread among the seedlings in a nursery.

Bitancourt (10) in 1935 published a short note describing a mosaic of papaya observed on plantations in São Paulo, Brazil. It is characterized by vein chlorosis of the older leaves and in the young leaves by malformation, stunting, reduction of the leaf blade, and by leafroll. Goncalves-Silva (18) in 1941 also reported mosaic as one of the diseases of papaya in Brazil and stated that it may sometimes be confused with the effects produced by mites on papaya.

An undescribed papaya mosaic was reported by Mueller (37) in 1941 to be extremely severe in Venezuela except in the Andes region.

An undescribed mosaic disease of papaya was reported for the first time from Rhodesia in 1940 by Hopkins (23).

In India at least two distinct papaya viruses have been reported. In 1939 Thomas and Krishnaswami (51) described a leaf-crinkle disease found at Coimbatore in South India. Instead of growing erect, the stem acquires a zigzag appearance. The leaves are crinkled and the margins curve downward and inward simulating an inverted cup. Plants which become diseased while small grow without appreciable stunting and retain symptoms throughout life. The disease was transmitted by grafting, symptoms developing in 120 days.

In 1940 a disease called papaya leaf curl was reported from the Madras Presidency (8). It was transmitted to healthy plants by grafting.

In 1946 Sen *et al.* (44) described a leaf-curl disease of papaya from Bihar as follows: "A serious disease of the papaya initiating with the symptoms of etiolation and curling of leaves, in advanced stages showing crippled leaves and fruits of various degrees and ultimately bringing about premature death of the plant has been observed." The disease was not seed-borne, but was juice-transmissible. The report asserts further that the disease could be initiated independent of inoculation by waterlogging the soil in which the papayas grow. However, symptoms resulting from growth in waterlogged soil were undoubtedly not due to a virus.

Su (50) in 1934 reported a papaya disease in Burma, considered to be due to a virus, which causes crinkling and distortion of the leaves and in severe cases adversely affects the fruits.

A virus disease of papaya was reported to occur in Kwangtung Province, China, by Tu (52) in 1932 and by Ho and Li (20) in 1936. Affected leaves are curled and stunted, and bear chlorotic patches between the veins. The margin of the inter-veinal lamina is often deeply indented. Frequently the trees are defoliated except for a few leaves at the top.

Yellow crinkle of papaya was reported from Queensland, Australia, by Morwood (36) in 1931, Simmonds (45, 46) in 1934, 1936, and 1937, and by Da Costa (15) in 1944. This disease causes the older leaves to turn yellow and droop. Between the veins of young leaves thin, translucent areas develop and later drop out. As these leaves mature they remain light yellowish green and appear crinkled. The tips of the segments of very young leaves curl downward and inward. In advanced stages the tree is bare of leaves except for a few stunted, distorted leaves at the apex. Yellow crinkle has not been transmitted mechanically or by insects.

A die-back disease of papaya was described from Java in 1931 by Rant (42), who reported bacteria as the causal agents. The symptoms resemble somewhat those caused by virus in some regions of the world, and Brierley (11) listed it as a virus disease in 1945.

Parris (39, 40, 41) from 1938 to 1941 discussed a new papaya virus disease discovered at Waialua and Lualualei, Oahu, in 1937. Mechanical transmission was obtained in over 75 per cent of the plants inoculated. Symptoms appeared in from 16 to 21 days after inoculation. Diseased plants are stunted and the leaves become chlorotic. The petioles of dis-

eased leaves bend down from their point of attachment on the stem. Hydrotic streaks occur on the petioles of diseased leaves and on the main stem. These streaks are linear, dark green, and slightly raised. Diseased leaves fall in four to six weeks after first symptoms, the stem is left bare except for a few stunted, distorted leaves at the apex and frequently a fringe of lower leaves which developed prior to symptom expression. Fruits on diseased trees "bleed" profusely. The incidence of this disease gradually diminished in the field and it has been observed only occasionally since 1939. Holdaway and Look (21) in 1940 reported negative results from preliminary vector experiments involving 8 species of insects.

Papaya ringspot disease, discussed in the succeeding pages of this paper, is the second papaya virus disease to be described from Hawaii. The restricted distribution of the disease on Oahu and its rapidity of spread within infected plantings, coupled with its deleterious effect upon commercial acreages at Kailua, suggest that it has been established in papaya in Hawaii for a relatively short time. The origin of the disease and its possible occurrence on islands or continental areas outside of Hawaii are not known.

MATERIALS AND METHODS

All papaya plants used in this investigation were of the Solo variety.

Experimental plants varied in age from 4 weeks to 15 months and in height from 2 inches to 15 feet at the time of inoculation. Test plants were grown in the greenhouse in cans varying in size from one pint to one gallon. Papayas placed in the field were usually from 6 to 20 inches tall at the time they were transplanted. In experimental field plots the plants were spaced either $2\frac{1}{2}$ or 3 feet apart in the row with 3 feet between rows. In commercial plantings the trees are usually spaced 10 to 12 feet apart.

At least one check tree was maintained for every tree used in a transmission test. In the greenhouse no control plants acquired the disease. In the field, natural spread to control plants occurred in all plots containing diseased trees if the plots were maintained for several months. Symptoms on the first control plants to become infected by natural spread usually appeared in 4 to 6 weeks after the first series of experimentally infected trees in the plot developed symptoms. When plots began to show evidence of natural spread of the disease they were not used further in transmission experiments.

PRIMARY FOLIAGE SYMPTOMS

The symptoms of papaya ringspot disease occur in the foliage, fruit, and main stem of the plant. Considerable variation exists in the degree of symptom expression.

The first evidence of the disease in plants inoculated with ringspot virus by means of *Myzus persicae* is a puckering or bulging of the leaf tissue between the secondary veins and veinlets on the upper surface of the young terminal leaves (Fig. 1). A tendency to roll downward and inward is

also apparent on the margins and distal points of the leaves. These symptoms, which usually appear in two weeks, are particularly pronounced in young plants growing rapidly in the field. As the affected leaves become larger, the puckering becomes less conspicuous and the leaves acquire a mildly rugose appearance. This is due to small, scattered convexities and concavities formed by the leaf surface between the smaller veins. Frequently most of these blister-like bulges are produced on the lower surface of the leaves, leaving portions of the upper surface pitted. This is especially true on plants which have had the disease for several months.

As they approach maximum expansion, affected leaves on field trees show a distinct tendency to roll upward along the margins. This is most

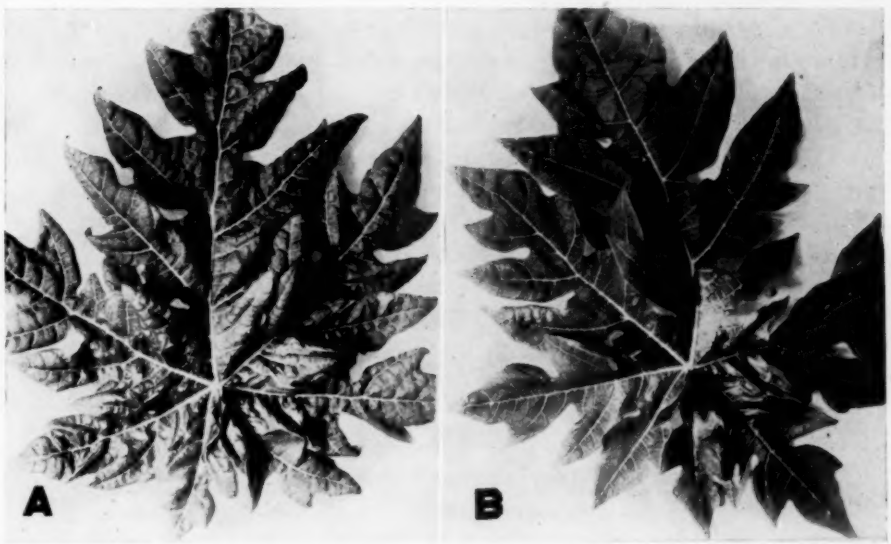

FIG. 1. A. Primary symptoms of papaya ringspot in young leaf of field tree, showing puckering of tissue between veinlets and downward rolling of margins and tips of leaflets. B. A healthy leaf of approximately the same age.

conspicuous on trees which have been diseased for several months. This characteristic is in contrast to that of young diseased leaves to roll downward and inward along the margins.

The general color of affected leaves is lighter green than normal. Moreover, the green color of the interveinal tissue is not of uniform quality but is characterized by irregular areas which are lighter green than the rest of the leaf. These lighter areas occur primarily along the smaller veins and veinlets but frequently extend throughout the interveinal tissue.

FACTORS AFFECTING PERIOD OF DEVELOPMENT OF PRIMARY SYMPTOMS

The time necessary for ringspot virus to produce primary foliage symptoms in papaya varies from 9 to 39 days after inoculation. An incubation period exceeding 21 days is rare, however.

The most important factor determining the length of the incubation period is the growing condition of the plant. However, the site of inoculation also has a definite though less marked effect. Plants making rapid, vigorous growth typically express symptoms in from 9 to 14 days when inoculated by means of aphids. Plants whose growth, at the time of inoculation, has been arrested or severely retarded by growing too long in small cans usually require a longer time for symptom production. In one experiment involving 8 young plants making rapid growth in gallon cans in the greenhouse, the incubation period varied from 9 to 15 days with an average of 10.5 days. In another experiment 31 plants, 8 to 10 inches tall and making slower growth in quart cans, developed symptoms in from 11 to 21 days with an average of 15 days.

An experiment was conducted to determine the effect of inoculation site on incubation period of the disease in field papaya trees 3 to 4 feet in height. Forty-four trees were infected by means of *Myzus persicae* caged on leaves of varying maturity.

Plants inoculated at the growing point and in the youngest three expanding leaves produced symptoms in from 10 to 15 days with an average of 11.1 days. Plants inoculated in the 4th to 11th youngest leaves developed symptoms in from 12 to 20 days with an average of 14.6 days. There was no difference in incubation period between plants inoculated in the 4th youngest leaf and those inoculated in the 10th or 11th youngest leaves. Sixty noninoculated check plants scattered throughout the plot remained disease-free.

MOSAIC OR SECONDARY FOLIAGE SYMPTOMS

Marked mosaic or mottle symptoms develop in the leaves of the tree canopy and in the small axillary leaves (Fig. 2). The development of this symptom requires a longer period of time than does the initial foliage symptom and is favored by the cool, cloudy weather common during the winter months in Hawaii. The leaf mottle results largely from accentuation of the differences in color intensity which are present but inconspicuous during the earlier stages of the disease. Reduced light seems to be a requirement for the production of prominent mottle symptoms.

During the winter months the mottle in the leaves is very marked; in contrast, during summer this character becomes poorly defined. However, diseased trees which were grown to a height of 9 and 10 feet under crowded field conditions ($2\frac{1}{2}$–3 feet apart) developed a striking mottle pattern in the shaded axillary leaves during the summer months even though the leaves of the canopy showed little or no mottle. Diseased plants, spaced far enough apart to permit more normal exposure of the axillary leaves to sunlight, developed only mild mottle in these leaves during the summer months.

The time necessary for the production of mottle symptoms is variable, depending to a large extent on the prevailing climatic conditions. The

shortest period recorded for the development of a strong mottle in the top foliage was 24 days after inoculation. The more typical time period during the winter months, however, is six weeks after inoculation. In contrast to this, plants inoculated during early summer did not develop even mild top mottle until three months after inoculation although the shaded axillary leaves on some of these plants had a well-defined mottle in 40 days.

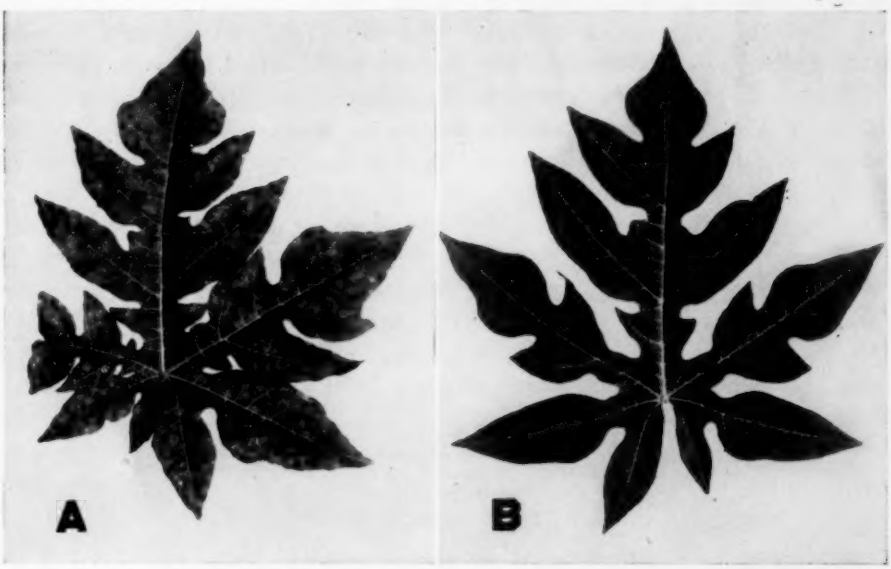

FIG. 2. A. Axillary leaf from a ringspot papaya tree, showing mosaic symptoms and leaf rugosity. The axillary leaves were shaded by the foliage canopy of trees growing close together. Mottle symptoms were produced in these leaves during the summer in the absence of well-defined mottle in the top leaves of the tree. B. Healthy axillary leaf, grown in shade, from a non-infected tree growing in the same plot as "A".

STEM SYMPTOMS

The main stem of papaya plants growing in small cans at the time of infection frequently develops dark green spots and streaks of an oily or water-soaked appearance. These are usually most common on the middle two-thirds of the stem. This symptom is characterized first by the development of a number of distinct, round spots approximately $\frac{1}{16}$ inch in diameter. These range in number from 4 to over 100 on the stem of a single plant 10 inches in height. In severe cases the spots increase rapidly in number and definition during the first few days after they become discernible. As they increase in number the water-soaked stem spots coalesce to form larger areas which frequently appear as elongate streaks.

The time required for the development of the dark green spots and streaks on the stem varies, depending apparently on the growing condition of the plant. In one experiment with 39 test plants (31 growing in quart cans and 8 in one-gallon cans), the shortest time recorded for the production of stem symptoms on plants infected by means of *Myzus persicae* was

13 days. The longest incubation period was 29 days. The average for the entire group was 17.7 days. Typically, the stem symptoms develop within a few days after the primary foliage symptoms have appeared. In several instances the foliage and stem symptoms appeared the same day. In 2 cases stem symptoms were recorded for plants which failed to develop recognizable foliage symptoms. Nine of the 39 trees produced foliage symptoms but failed to develop stem symptoms.

The stem symptom has not been observed on field trees which were well established and growing rapidly at the time of infection. It has, however, appeared on plants which were transplanted to the field from small cans within a few days after their inoculation by means of aphids. In these cases the plants made little growth during the first 10 days in the field. The water-soaked streaks persisted for several weeks.

Observations indicate that the stem-spotting symptom is correlated with severe retardation of growth in diseased plants. Papayas grown in small cans grow much slower than do plants in the field. It is typically under these conditions of slow growth that stem symptoms appear.

As a rule the plants which were making such poor growth in small cans that the foliage symptoms were indistinct or mild developed severe water-soaked spotting over the stems. Conversely, plants which produced the strongest foliage symptoms typically developed fewer and less conspicuous spots on the stems.

FRUIT SYMPTOMS

Papaya fruits show virus symptoms, as rings or spots, both during their early stages of development and as they approach maturity. The flavor and texture of infected fruits, however, are not impaired by the disease.

Young fruits, one to 3 inches long, frequently develop small, light green rings on their surface. The rings on young fruits are usually relatively few in number and inconspicuous. They become even less distinct as the fruits become larger and darker green. Young fruits bearing ring symptoms were marked and the position of the rings on the individual fruits was recorded. When these fruits became mature green and developed typical yellow rings, the position of the light green rings on the young fruits had no direct relation to the position of the yellow rings on the mature fruits. The rings on small young fruits were usually localized as one or 2 compact groups of 6 to 12 rings per group. The yellow rings produced on these fruits at maturity were numerous and were scattered over most of the fruit surface. Many of the fruits on experimentally infected trees developed no detectable rings or spots while the fruits were small. However, yellow rings invariably appeared on all of the fruits as they approached maturity.

The yellow spots and yellow rings with green centers (Fig. 3, B) on mature green fruits provide the most striking and reliable symptom of the disease. The size of the spots ranges from approximately $\frac{1}{16}$ to $\frac{1}{8}$ inch in

diameter. The rings vary in size from $\frac{1}{8}$ to $\frac{3}{4}$ inch in diameter. The number of spots or rings on a single fruit may vary from a few to over 150.

FACTORS DETERMINING TYPE OF FRUIT SYMPTOM

The developmental stage of a given fruit at the time the plant becomes infected with ringspot virus determines the type of symptom produced on that fruit.

Typically, fruits $\frac{5}{6}$-grown or older at the time of infection ripen without evidence of the virus. Fruits $\frac{2}{3}$-mature or somewhat younger at the time of infection develop yellow spots at the mature green stage. These appear in from 6 to 8 weeks after the tree becomes infected. Fruits maturing

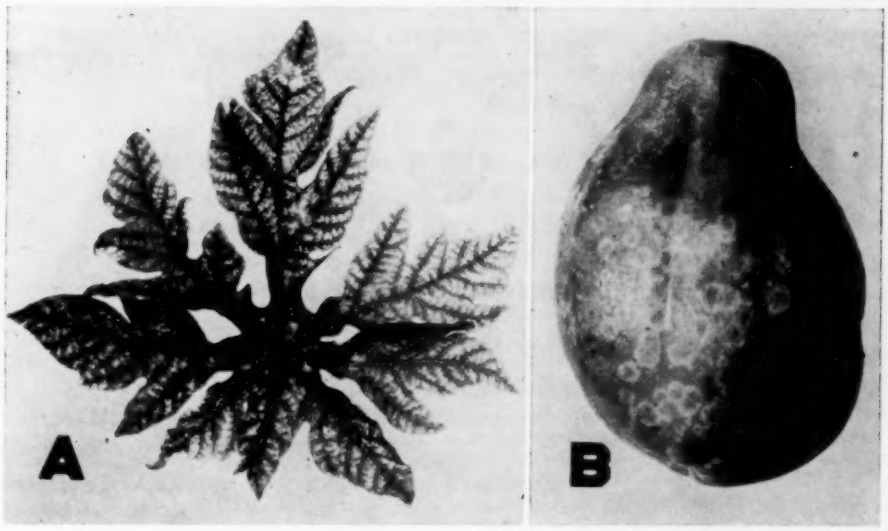

FIG. 3. A. Papaya leaf from a tree naturally infected with ringspot in a commercial field at Kailua, Oahu. Experimentally infected trees, receiving good care, have failed to develop such severe symptoms. B. Papaya fruit showing yellow rings with green centers caused by ringspot virus. This symptom appears a few days before the maturing fruit changes color from green to ripe yellow.

during the first 3 weeks after the first spotted fruit appears likewise exhibit yellow spots as the only disease symptom. During the next 3 to 5 weeks, maturing fruits develop both solid yellow spots of enlarging size and small rings, i.e., yellow spots with very small green centers. With increasing time the rings produced on newly ripening fruits become progressively larger and more numerous while the number of solid yellow spots decreases. Three months after the first yellow spots have appeared the symptom on maturing fruits has changed to a predominance of rings with only a few spots remaining. If no fruits have been formed, or the oldest fruits are not more than approximately $\frac{1}{2}$ grown at the time the plant acquires the disease, the first fruits to mature, as well as all mature fruits subsequently produced, carry well-defined rings and few if any spots.

Table 1 indicates the relationship of fruit development at the time of infection to the type of symptoms on maturing fruits. The time periods vary considerably from tree to tree and from season to season depending upon the growth rate. Two trees, infected during the winter and producing ripe fruit at the time of infection, required 47 and 61 days, respectively, after infection to produce the first spotted fruit and 71 and 82 days, respectively, to produce the first fruit with rings.

TABLE 1.—*Effect of stage of fruit development at time of infection on symptoms on maturing fruits*

Approximate stage of fruit when plant was infected.[a]	Symptoms on maturing fruits	Approximate time from infection to fruit maturity[a] (in days)
5/6 grown	No symptoms	25
2/3 grown	Small, solid yellow spots only. No rings.	50
1/2 grown	Predominantly solid yellow spots; a few small, distinct rings.	75
1/3 grown	Rings common and enlarging; solid yellow spots still in majority.	100
1/6 grown	Predominantly well defined rings; only a few solid yellow spots.	125
Newly fertilized flowers	Rings only	150

[a] The figures listed fluctuate from tree to tree and vary with the season. Dr. Wm. B. Storey, Horticulturist at the University of Hawaii, has informed the writer that in Manoa Valley, where these tests were conducted, the average time for papaya fruits to reach the firm ripe stage of maturity after pollination is 150 days. Time is shorter than 150 days for fruits starting development during March and April and longer for those starting during November and December.

EFFECT OF PAPAYA RINGSPOT VIRUS ON PLANT GROWTH AND VIGOR

In addition to the rugosity and mosaic mottle produced in papaya leaves, ringspot virus also causes a reduction in size of the leaf lamina and in length of the petiole. The tree as a whole is also stunted by the disease. The degree of stunting, however, varies considerably. In the experimental plots of the University of Hawaii, at Honolulu (on the lee side of the island of Oahu), where the plants were fertilized and irrigated regularly, papayas, experimentally infected when 10 inches high, grew moderately well and produced fruit despite the presence of the disease. These plants were not, however, as large and vigorous as were the non-diseased trees. In the commercial papaya plantings in the vicinity of Kailua, on the windward side of Oahu, however, effects of the disease are much more severe and infected trees usually become commercially unprofitable within a year or two after becoming infected. In these plantings adverse environmental conditions, such as seasonal drought, affect ringspot trees more severely

than non-infected trees. Stunting of all parts of the tree becomes extreme and the loss of chlorophyll in diseased foliage frequently leaves much of the interveinal lamina translucent (Fig. 3, A).

Ringspot virus has no effect on the normal flow of latex from wounds made in stem, leaf, or green fruit tissue.

RATE OF VIRUS MOVEMENT THROUGH PAPAYA PLANT

Ringspot virus moves rapidly upward through the branches and main stem of the plant. Symptoms typically appear in the terminal foliage 13 or 14 days after the plants are inoculated on mature leaves. The incubation periods of Hawaiian papaya mosaic or Waialua disease (16–21 days) and of mosaic in Puerto Rico (8–21 days) are also short.

The rate of virus movement downward in papaya plants has received little investigation. Smith (47) in 1932 and Edwards (16) in 1933, reporting on the papaya mosaic in Jamaica, stated that a reasonable proportion of "recoveries" could be obtained if diseased trees were cut back to the soil level as soon as the first symptoms of the disease appeared. Furthermore, in some cases the lower branches remained healthy if the diseased main stem, dying with the disease, were removed.

The Waialua disease of papaya in Hawaii, described by Parris (39), sometimes killed the growing point without invading the branches later produced lower down the tree. Moreover, if diseased trees were decapitated, the new growth developing from the basal portion of the stem was healthy or some shoots were healthy and some diseased.

In Trinidad, Baker (9) in 1939 observed that the top of mosaic-diseased trees usually died in 8 or 9 weeks after symptoms appeared. This was followed by secondary rotting which stopped 1 or 2 feet below the original apex of the tree. A large number of axillary buds were subsequently produced below the dead point. The upper buds became diseased at once but frequently the buds lower down the tree grew to large size and produced fruit.

These results suggest that some papaya viruses are slow in moving downward through the main stem of the plant.

The present studies of papaya ringspot disease did not include decapitation of large, recently infected papaya plants to determine how rapidly the virus invaded the lower portions of the main stem. Several plants, infected when one foot or less in height, which had been diseased for 3 or 4 months, were cut off at or near the ground level. The new shoots produced from the base of these plants were all diseased.

A single preliminary experiment was conducted to determine the time required for virus to move from the point of inoculation in one branch of a dichotomously branched papaya to the other branch and there to produce symptoms. Although only 2 plants were available for use in the test, the results justify brief mention.

Two papaya plants 3½ feet tall were cut off at a point 8 inches above the ground on February 28, 1946. Each plant subsequently produced 2 new lateral shoots which arose on opposite sides of the main stem. One branch on each plant originated at a point 3 inches above the point of attachment of the second branch. On April 6, 1946, when the new shoots were each approximately 14 inches long, 100 infective *Myzus persicae* were caged on the terminal of one branch on each tree. Symptoms appeared in the new growth of each of the inoculated branches in 12 days. The terminal growth on the noninoculated branch of each of the 2 trees did not develop symptoms until 38 and 44 days, respectively, after the trees were initially inoculated. The original inoculation was made on the upper branch of one plant and on the lower branch of the other.

These results indicate that ringspot virus movement is relatively slow downward through the branch of a rapidly growing papaya plant. In these 2 tests when the virus moved down a branch and into the main stem in order to enter a non-infected shoot on the same plant and there produce symptoms, a time period was required which was approximately 3 times as long as the incubation period in the inoculated branch.

FAILURE TO INFECT OTHER SPECIES OF PLANTS

Extensive host range studies have not been reported for any of the papaya viruses listed in the literature. The botanical affinities of the genus *Carica* are not well known. According to Storey (49) papaya, now placed in the Caricaceae, has at one time or another been placed in the following different families: Passifloraceae, Cucurbitaceae, Bixaceae, and Papayaceae.

The native home of the papaya is considered to be Central America, the British West Indies, or Mexico. No closely related plants are known to occur in Hawaii, the passion flower vine, *Passiflora* spp., possibly being the nearest in botanical relationship.

Experiments were conducted in Hawaii to determine whether papaya ringspot virus could be transmitted by means of aphids to hosts other than papaya. Nearly all of the test plants used were grown from seed in the greenhouse and were fed upon by infective aphids while the plants were still small. Three weeks after inoculation, non-infective *Myzus persicae* were placed on the test plants, allowed to feed for 24 hours, and then transferred to healthy papaya seedlings.

Table 2 lists the plants used in the host range investigation. Twelve plant families were tested. These were represented by 16 species of plants, most of which occur commonly in the papaya ringspot disease area. Virus symptoms failed to develop in any of the plants tested and previously non-infective aphids failed to recover ringspot virus from 11 species used in recovery tests.

It is of further interest that of the papaya viruses reported in the literature, none is known to infect other hosts.

VIRUSES TESTED ON PAPAYA

A number of virus diseases occur in cultivated and wild plants in the papaya ringspot area of Oahu. With the exception of the mosaic diseases of pepper, Commelina, and Crotalaria this is the first report in the literature of the diseases discussed below occurring in Hawaii. Experiments were conducted to determine if any of these diseases could be transmitted

TABLE 2.—*Plant species tested as possible hosts of papaya ringspot virus. Inoculations were made by means of infective Myzus persicae. All tests were negative.*

Plant family	Test plant	Number of plants inoculated	No. aphids used per test
Commelinaceae	*Commelina diffusa* Burm. (Wandering Jew)	3	100
Crassulaceae	*Bryophyllum calycinum* Salisb.	2	150
Chenopodiaceae	*Beta vulgaris* L. (Table beet)	49	30
Compositae	*Lactuca sativa* L. (Lettuce)	27	20
Cucurbitaceae	*Cucumis sativus* L. (Cucumber)	20	100
Cruciferae	*Brassica chinensis* L. (Pakchoi)	72	50
Malvaceae	*Malvastrum coromandelianum* (L.) (False mallow)	4	50
Nyctaginaceae	*Mirabilis jalapa* L. (Four o'clock)	12	50
Leguminosae	*Crotalaria incana* L. (Fuzzy rattle pod)	9	100
Passifloraceae	*Passiflora foetida* L. (Passion flower)	2	30
	P. pfordti (Passion flower) (= *alatocaerulea*)	3	100
Portulacaceae	*Portulaca oleracea* L. (Purslane)	20	75
Solanaceae	*Lycopersicon esculentum* Mill. (Tomato)	21	50
	Capsicum frutescens L. (Pepper)	13	50
	Solanum tuberosum L. (Potato)	10	500
	Nicotiana tabacum L. (Turkish tobacco)	21	200
		288	

to papaya by means of aphid vectors, and in particular to determine if any produce symptoms in papaya which resemble those of ringspot disease.

The literature contains no report of papaya having been experimentally infected with any virus occurring in another plant species. None of the viruses tested during this study produced symptoms in papaya and in no instance were the experimental viruses recovered from papaya.

Myzus persicae was used in all tests except those involving Crotalaria mosaic. In the Crotalaria tests, *Aphis gossypii* was used. This species also constituted part of the aphid population in tests with Commelina mosaic and with pepper mosaic.

Hibiscus ringspot.[3] Several hibiscus plants in Honolulu were found

[3] Brought to the attention of the writer by Dr. F. O. Holmes of the Rockefeller Institute for Medical Research.

to carry ringspot symptoms on the leaves. The rings, approximately $\frac{1}{8}$ of an inch in diameter, are lighter green than normal tissue and contain dark green centers. On some plants the rings are very distinct; on others they are scattered throughout a more diffuse mosaic area and are less conspicuous. The hibiscus flowers on some plants on Oahu and Kauai also carry a large diamond-shaped pattern of breaking in the color of the petals. Whether this is caused by the same virus producing ringspots on the leaves has not been determined.

Scaevola frutescens ringspot. This plant is known in Hawaii as beach "naupaka." A virus disease of *S. frutescens* was first noted June 21, 1946, in the Kailua area of Oahu.[4] The symptoms are very variable and include chlorotic, etched ring and oak leaf patterns and mottling and crinkling of the leaves. On some leaves, rings occur in a concentric pattern. This disease was subsequently observed on the island of Kauai where no papaya ringspot is known to occur.

Passiflora foetida mosaic.[3] Conspicuous mosaic symptoms occur in the leaves of this plant in Honolulu.

Passiflora pfordti mosaic. This plant, a hybrid resulting from crossing *P. alata* Dry and *P. caerulea* Linn., carries virus symptoms in Honolulu.[5] The younger leaves bear small yellow spots while the symptoms in older leaves appear as irregular areas of light green tissue.

Commelina diffusa mosaic. This disease, caused by a cucumber mosaic virus, is very common in Hawaii.

Stachytarpheta cayennensis mosaic. This disease of false vervain, characterized by an irregular mosaic pattern in the leaves, was found at Kailua, Oahu, in the papaya ringspot disease area.

Pepper mosaic. This disease is common in Hawaii but the identity of the virus or viruses involved has not been determined.

Crotalaria mosaic. This virus is common on *Crotalaria incana* L. and *C. mucronata* Desv. in the Hawaiian Islands. It causes severe dwarfing of the plant as well as mosaic and malformation of the leaves.

DISCUSSION

Although the papaya is native only to the American tropics (49), during the seventeenth and eighteenth centuries it spread throughout the tropical and sub-tropical regions of the world. As the distribution of papaya was accomplished largely by means of seed rather than by the shipment of growing plants and since no papaya virus has yet been found to be seed-transmitted, it is probable that the virus diseases known to occur in widely separated regions of the world several years ago did not originate in the same locality. The modern plane, however, makes possible the transportation of virus-infected plant parts or of infective insect vectors between all of the papaya-growing areas of the world.

[4] This disease was brought to the attention of the writer by Dr. M. B. Linford of the Pineapple Research Institute of Hawaii.

[5] This disease was brought to the attention of the writer by Dr. R. C. Lindner, formerly of the University of Hawaii, now at Washington State College.

Virus diseases of papaya have been reported from Cuba, Jamaica, the Dominican Republic, Puerto Rico, Tobago, Trinidad, Venezuela, British Guiana, Brazil, Rhodesia, India, Burma, China, Australia, and Hawaii. In addition, a papaya disease characterized by symptoms similar to those caused by some viruses occurs in Java. In 1931 this disease was reported to be due to bacteria. Despite the extreme separation of some of the above regions, several of the described viruses have certain symptoms in common. This is true even in the case of diseases such as bunchy top of Puerto Rico and Waialua disease of Hawaii which are known to be caused by fundamentally distinct viruses. Both diseases typically denude the tree except for a tuft of leaves at the top and both cause water-soaked streaks on the stems and petioles. However, bunchy top is a persistent leafhopper-transmitted virus which is not mechanically transmissible, whereas Waialua disease was readily transmitted by means of plant juice. It is evident, therefore, that the co-identity or close relationship of viruses of papaya from widely separated areas cannot be assumed merely because some of the symptoms are similar. More complete knowledge regarding their vectors and properties as well as their symptoms under various environmental conditions must be acquired before relationships can be inferred.

Some of the symptoms which figure prominently in the descriptions of many known papaya virus diseases are: (1) bunchy top, or the defoliation of the plant except for a few leaves at the top, (2) die-back at the top, (3) curling and distortion of the leaves, (4) water-soaked or oil-like streaks on the petioles and stems, (5) fruit symptoms, and (6) abnormal latex flow when the tree is wounded.

The bunchy top symptom is reported for bunchy top of Puerto Rico, bunchy top (Mosaic type A) and Cotorro mosaic (Mosaic type B) of Cuba, curly leaf of Santo Domingo, yellow crinkle of Australia, mosaic or curl of China, and Waialua disease of Hawaii. In addition, water-logged soil and other adverse environmental conditions may cause a similar symptom.

Die-back is reported to be characteristic of the Trinidad mosaic and also of the die-back disease of Puerto Rico. In addition, Baker (9) speaks of the mosaics of Jamaica and Santo Domingo as die-backs similar to that of Trinidad.

Leaf-curl symptoms and other leaf distortions have been reported from British Guiana, Brazil, Santo Domingo, Cuba (Cotorro mosaic), Puerto Rico (Mosaic), India, Burma, China, and Australia.

Water-soaked areas on the stem, petiole, or both are reported for bunchy top, die-back and mosaic of Puerto Rico, diseases A (bunchy top) and B (Cotorro mosaic) of Cuba, Trinidad mosaic, Waialua mosaic and ringspot of Hawaii.

Bunchy top and die-back of Puerto Rico and bunchy top of Cuba reduce or stop the flow of latex from wounds, while, according to Adsuar (4), in Puerto Rico "this phenomenon is much less common in the mosaic type of disease." Latex flows freely from the wounded tissue of plants in-

fected with Cuban Cotorro mosaic. In Hawaii the latex response of trees carrying ringspot disease is normal whereas, according to Parris (41), Waialua disease causes the fruits to bleed profusely.

Fruit symptoms have been reported for only a few papaya diseases. Yellow spots and rings are caused by ringspot virus in Hawaii and green or dark brown rings are produced by mosaic of Puerto Rico. Spots sometimes appear on the fruit of plants having Cotorro mosaic of Cuba.

The co-identity of any two or more papaya virus diseases must still be established. It is probable, however, that the bunchy top disease of Puerto Rico and type A disease of Cuba are the same. The existence of this disease on other islands of the Caribbean is highly plausible.

Adsuar (4) considers Cotorro mosaic of Cuba to be identical with or closely related to the aphid-transmitted mosaic of Puerto Rico. However, the stem and fruit symptoms reported from Cuba are different from those described from Puerto Rico.

Papaya ringspot disease of Hawaii and papaya mosaic of Puerto Rico resemble each other in that they are both juice-transmissible, both have aphids as their vectors, and in both diseases the time required to produce symptoms is essentially identical. They differ in leaf and fruit symptoms. Ringspot causes a mild-to-marked mosaic mottle in the leaves without conspicuous leaf deformation. The fruit symptoms consist of bright yellow spots and rings at the mature green stage. In Puerto Rico papaya mosaic causes extreme malformation of the leaf and reduction of the lamina to a filiform structure. Green and dark brown rings are produced on the fruit but the stage of fruit maturity at which the rings appear has not been published. The two virus diseases known from Hawaii, Waialua disease and ringspot, are distinct in their reported symptoms.

The reports of papaya virus transmission are relatively few. Mechanical transmission has been announced for Waialua disease and ringspot in Hawaii, mosaic of Puerto Rico, leaf curl of Bihar, India, and curly leaf of Santo Domingo. The latex transmission of curly leaf reported by Ciferri (13) in Santo Domingo needs confirmation. Graft transmission has been reported for leaf crinkle at Coimbatore, South India, and for papaya leaf curl in the Madras Presidency of India.

The only known leafhopper vector of a papaya virus is *Empoasca papaya* Oman which transmits bunchy top in Puerto Rico. Ringspot virus in Hawaii was the first papaya virus to be experimentally transmitted by aphids. Puerto Rico mosaic has also been found to be transmitted by an aphid.

In general, virus movement in plants is believed to be associated with the translocation of food. Hence, viruses move rapidly downward in many plants. From the information available in the literature and from an experiment on papaya ringspot it is concluded that downward movement of papaya viruses is relatively slow. The papaya is a rapidly growing, herbaceous, tree-like plant which has no dormancy. As the plant grows the

lower leaves fall and new leaves and flowers are produced at the top. Perhaps the direction of food translocation, therefore, is predominantly between the leaves and fruit in the upper part of the tree with relatively little going from the foliage canopy to the roots. This could reduce the opportunity for virus to become established in the lower part of the plant and might account for the fact that healthy shoots may be produced from the lower parts of papayas infected with viruses such as Trinidad mosaic and Waialua disease. Experiments with papaya ringspot indicate that virus movement upward is rapid in the plant but that downward movement is slow.

SUMMARY

A review is given of the symptoms and transmission reports of papaya viruses recorded from Cuba, Jamaica, Dominican Republic, Puerto Rico, Tobago, Trinidad, Venezuela, British Guiana, Brazil, Rhodesia, India, Burma, China, Australia, and Hawaii.

Papaya ringspot virus, known only from the island of Oahu, T. H., causes a typical mosaic disease in papaya. The virus is carried by aphids and is mechanically transmissible.

Symptoms are produced in the foliage, fruit, and stems of the plant. Rugosity and mottle, without marked distortion, characterize the leaf symptoms. Reduced light enhances the expression of leaf mottle. Yellow spots and yellow rings with green centers are produced on diseased fruits at the mature green stage. The stage of fruit development at the time of infection determines the type of fruit symptom. Fruits infected when young develop yellow rings at maturity while fruits $\frac{2}{3}$-grown when infected produce only solid yellow spots. Small, inconspicuous, light green rings sometimes also occur on very young fruits of diseased plants.

Young plants making slow growth when infected sometimes develop water-soaked spots and streaks on the stem.

Primary foliage symptoms usually appear in from 9 to 21 days after infection. Stem symptoms typically take a few days longer to develop than do foliage symptoms.

Plants inoculated in the terminal growth by means of infective aphids develop symptoms a few days earlier than do plants inoculated in the older leaves.

Papaya ringspot virus was shown to move rapidly upward through the papaya plant, but downward movement of the virus was found to be relatively slow.

During experiments to determine the possible host range of papaya ringspot virus, sixteen species of plants representing 12 plant families were inoculated by means of infective aphids. All test plants remained healthy. Non-infective aphids failed to recover virus from any of the 11 plant species used in attempts to recover virus from the symptomless inoculated test plants.

Efforts to transmit 8 virus diseases, occurring in other hosts in Hawaii, to papaya were unsuccessful.

UNIVERSITY OF HAWAII AGRICULTURAL EXPERIMENT STATION,
HONOLULU, T. H.,
AND
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

LITERATURE CITED

1. ACUNA, J., and F. ZAYAS. Fruta bomba o papaya. Revista de Agr. (Cuba) **23**: 49–80. 1940.
2. ———— and ————. El mosaico y otras plagas de la fruta bomba (*Carica papaya* L.) Estac. Exp. Agron. Circ. 85. 1946.
3. ADSUAR, JOSE. Transmission of papaya bunchy top by a leafhopper of the genus *Empoasca*. Science **103**: 316. 1946.
4. ————. Studies on virus diseases of papaya (*Carica papaya*) in Puerto Rico. I. Transmission of papaya mosaic. Univ. Puerto Rico Agr. Exp. Sta. Tech. Paper No. 1. 1946.
5. ————. Studies on virus diseases of papaya (*Carica papaya*) in Puerto Rico. II. Transmission of papaya mosaic by the green citrus aphid (*Aphis spiraecola* Patch). Univ. Puerto Rico Agr. Ext. Sta. Tech. Paper No. 2. 1946.
6. ————. Studies on virus diseases of papaya (*Carica papaya*) in Puerto Rico. III. Property studies of papaya mosaic virus. Univ. Puerto Rico Agr. Exp. Sta. Tech. Paper No. 4. 1946.
7. ALVAREZ, L. A. Studies on the virus disease complex in the papaya. Ann. Rept. Univ. Puerto Rico Agr. Exp. Sta. (Rio Piedras) 1940–1941: 62. 1941.
8. ANONYMOUS. Report on the operations of the Dept. of Agr. Madras Presidency for year 1938–1939: 35–38. 1940.
9. BAKER, R. E. D. Papaw mosaic disease. Trop. Agr. (Trinidad) **16**: 159–163. 1939.
10. BITANCOURT, A. A. Mosaico do mamoeiro. O Biologico **1**: 41. 1935.
11. BRIERLEY, P. Viruses described primarily on ornamental or miscellaneous plants II. U. S. Dept. Agr. Plant Dis. Reptr. Suppl. 158: 168–200. 1945.
12. CASTELLI, ENRICO. Conclusions arrived at after thorough investigation on the cultivation of the papaw and its industrial application. Proc. Agr. Soc. Trinidad and Tobago **29**:25–32; 77–85; 129–131; 165–170; 219–220. 1939.
13. CIFERRI, R. Phytopathological survey of Santo Domingo, 1925–1929. Jour. Dept. Agr. Puerto Rico **14**: 5–44. 1930.
14. COOK, M. T. New virus diseases in Porto Rico. (Abstr.) Phytopath. **21**: 124. 1931.
15. DA COSTA, E. W. B. Diseases of the papaw. Queensland Agr. Jour. **58**: 282–293. 1944.
16. EDWARDS, W. H. Plant diseases in Jamaica in 1932. Rept. Gov. Entomologist. Ann. Rept. Dept. Agr. Jamaica 1932: 16–18. 1933.
17. GOENAGA, ALVARO. Notes on diseases of tobacco observed in Puerto Rico during the 1944–45 season. U. S. Dept. Agr., Plant. Dis. Reptr. **29**: 311–314. 1945.
18. GONCALVES-SILVA, S. Doencas do mamoeiro. O Biologico **7**: 220–225. 1941.
19. HENDRIX, J. W. and M. MATSUURA. Papaya ringspot. Univ. Haw. Agr. Exp. Sta. Biennial Rept. 1944–1946: 103–104. 1947.
20. HO, W. T. H., and L. Y. LI. A virus disease of papaya (*Carica papaya* L.). *In:* Preliminary notes on the virus diseases of some economic plants in Kwangtung Province. Lingnan Sci. Jour. **15**: 67–78. 1936.
21. HOLDAWAY, F. G., and WM. C. LOOK. Possible vectors of virus disease of papaya. Ann. Rept. Univ. Haw. Agr. Exp. Sta. for 1939: 37–38. 1940.
22. HOLMES, F. O., J. W. HENDRIX, W. IKEDA, D. D. JENSEN, R. C. LINDNER, and W. B. STOREY. Ringspot of papaya in the Hawaiian Islands. Phytopath. **38**: 310–312. 1948.
23. HOPKINS, J. C. F. Annual report of the senior plant pathologist for the year ending 31st December, 1939. Rhodesia Agr. Jour. **37**: 411–423. 1940.
24. JENSEN, D. D. Virus diseases of plants and their insect vectors with special reference to Hawaii. Proc. Haw. Ent. Soc. **12**: 535–610. 1946.

25. ————. A new virus disease of papaya. Univ. Haw. Agr. Exp. Sta. Biennial Rept. 1944–1946: 67. 1947.
26. ————. Papaya ringspot virus and its insect vector relationships. Phytopath 39: 212–220. 1949.
27. JENSEN, J. H. Studies of papaya bunchy top. Rept. Puerto Rico Exp. Sta. (Federal) for 1937: 82–87. 1938.
28. ————. Papaya bunchy top studies. Rept. Puerto Rico Exp. Sta. (Federal) for 1938: 121–125. 1939.
29. KEVORKIAN, A. G. Papaya-disease investigations. Monthly Rept. Puerto Rico Exp. Sta. (Federal) for January 1938. 1938.
30. LARTER, L. N. H. Papaw selection. Ann. Rept. Dept. Sci. and Agr. Jamaica for 1934: 33. 1935. 1935: 86. 1936. 1936: 71. 1937. 1937: 76–77. 1938.
31. ———— and MARTYN, E. B. A preliminary list of plant diseases in Jamaica. Mycological Papers No. 8. Imperial Myc. Inst. 1943.
32. LINDNER, R. C., D. D. JENSEN, and W. IKEDA. Ringspot: New Papaya Plunderer. Haw. Farm and Home 8 (10): 10, 12, 14. 1945.
33. ————, W. IKEDA, and M. AWADA. Papaya ringspot. Univ. Haw. Agr. Exp. Sta. Biennial Rept. 1944–1946: 118–119. 1947.
34. MARTYN, E. P. Papaw curly leaf. Divis. Rept. Dept. Agr. Brit. Guiana 1932: 119. 1934.
35. ————. Diseases of plants in Jamaica. Jamaica Dept. Sci. and Agr. Bul. No. 32 (N.S.): 1–32. 1942.
36. MORWOOD, R. B. The work of the pathological branch. Ann. Rept. Queensland Dept. Agr. and Stock 1930–1931: 47–48. 1931.
37. MUELLER, A. S. El reconocimiento de las enfermedades de las plantas cultivadas en Venezuela, 1937–1941. Boletin de la Soc. Venezolana de Ciencias Naturales 7: 99–113. 1941.
38. OMAN, P. W. New Eupterygine leafhoppers from Puerto Rico. Jour. Agr. Univ. Puerto Rico 21: 567–571. 1937.
39. PARRIS, G. K. A new disease of papaya in Hawaii. Amer. Soc. Hort. Sci. 36: 263–265. 1938.
40. ————. Virus disease of papaya. Rept. Haw. Agr. Exp. Sta. 1938: 34. 1939. 1939: 69. 1940. 1940: 67–78. 1941.
41. ————. Diseases of papaya in Hawaii and their control. Papaya production in the Hawaiian Islands. Haw. Agr. Exp. Sta. Bul. 87: 32–44. 1941.
42. RANT, A. Ueber eine Bakterienkrankheit bei dem Melonenbaum (*Carica papaya* Linn.) auf Java. Zentralb. Bakt. II, 84: 481–487. 1931.
43. SEIN, F., and J. ADSUAR. Transmission of the bunchy top disease of papaya (*Carica papaya* L.) by the leafhopper *Empoasca papayae* Oman. Science 106: 130. 1947.
44. SEN, P. K., B. D. GANGULY, and P. C. MALLIK. A note on a leaf-curl disease of the papaya (*Carica papaya* Linn.). Indian Jour. Hort. 3: 38–40. 1946.
45. SIMMONDS, J. H. Ann. Rept. Dept. Agr. and Stock, Queensland 1933–1934: 67–70. 1934. 1935–1936: 49–58. 1936.
46. ————. Diseases of papaw. Queensland Agr. Jour. 48: 544–552. 1937.
47. SMITH, F. E. V. Plant diseases in Jamaica in 1928. Ann. Rept. Dept. Sci. and Agr. Jamaica 1928: 19, 1929. 1930: 18, 1931. 1931: 17–20, 1932.
48. STELL, F. Papaw (*Carica papaya*) mosaic. Adm. Rept. Dir. Agr. Trinidad and Tobago, 1936: 56. 1937.
49. STOREY, W. B. The botany and sex relationships of the papaya. Papaya production in the Hawaiian Islands. Univ. Haw. Agr. Exp. Sta. Bul. 87: 5–22. 1941.
50. SU, M. T. Miscellaneous diseases. Rept. Operations Dept. Agr. Burma for year ended March 31, 1934: 31. 1934.
51. THOMAS, K. M., and C. S. KRISHNASWAMI. Leaf crinkle—a transmissible disease of papaya. Current Science 8: 316. 1939.
52. TU, C. Notes on some diseases of economic plants in South China. Lingnan Sci. Jour. 11: 489–506. 1932.

PAPAYA RINGSPOT VIRUS AND ITS INSECT VECTOR RELATIONSHIPS[1]

D. D. JENSEN[2]

(Accepted for publication November 1, 1948)

In 1946 and 1947 the writer (2, 3) reported that papaya ringspot virus had been transmitted experimentally in Hawaii by means of *Myzus persicae* (Sulzer) and by three unnamed *Aphis* species. In 1949 (4) experiments were reported, with *M. persicae* as the vector, which dealt with the symptoms and incubation periods of the disease in the foliage, stem, and fruit of the papaya. Data were also presented on the rate of virus movement through the papaya plant and on host range investigations of ringspot virus.

The present paper discusses the relationships found to exist between ringspot virus and *M. persicae,* the most important aphid vector. The identity, host preferences, and vector efficiency are also indicated for other species of aphids shown experimentally to transmit ringspot virus.

MATERIALS AND METHODS

The test plants (solo papaya) and experimental plots used in these investigations were described in a previous paper (4).

Populations of the green peach aphid, *Myzus persicae,* used in most of the vector tests, were reared on pakchoi (*Brassica chinensis*) in the greenhouse. Test insects were usually transferred individually by means of a fine camel's-hair brush. In some of the tests involving winged aphids, forceps were used to pick the insects up by the wings. In many of the tests using 150 or more aphids per test, transfers were made by placing portions of diseased leaves, infested with aphids, in the cage with the healthy test plant. In some of these tests direct contact between diseased and healthy foliage was prevented by placing the inoculum leaf on the soil an inch or two away from the test plant, or by placing the inoculum leaf on a piece of paper which rested on the foliage of the test plant. This precaution was found to be unnecessary, however, because in no instance did ringspot-infected leaves, in the absence of aphids, transmit the virus to healthy plants by contact.

The time of feeding on diseased and healthy test plants varied from 2 minutes to several days depending upon the type of experiment.

Two types of cage were used predominantly in this investigation. In most field tests a single leaf or the growing point of the plant was enclosed in a simple sleeve cage made of white organdy cloth with a weave of 88 threads to the inch. In the greenhouse the sleeve cage was replaced in most tests by a rigid rectangular cage. This was open at the bottom. Three

[1] Published with the approval of the Director as Technical Paper No. 178 of the University of Hawaii Agricultural Experiment Station.

[2] Formerly Assistant Entomologist, University of Hawaii Agricultural Experiment Station. Now Assistant Professor of Entomology and Assistant Entomologist, University of California, Berkeley, California.

sides and the top were covered with cloth and one side was covered with clear celluloid. This cage covered the entire plant and the bottom was thrust into the soil around the base of the plant. In a few greenhouse tests, single leaves of small plants were enclosed in sections of transparent sausage-casing, one inch in diameter, cut into sleeves of any desired length.

INSECT SPECIES TESTED AS VECTORS[3]

During the investigation of papaya ringspot disease, 9 species of aphids and one species of spider mite were caged in large numbers on papaya plants (Table 1). *Myzus persicae* was the only aphid species to survive for more than 2 weeks. The other species died out during the first 7 to 10 days on papaya despite the fact that some reproduction occasionally took place.

TABLE 1.—*Summary of papaya ringspot virus vector tests with aphids and red spiders*

Species	Individuals per plant	Number of tests	Number positive	Percentage transmission
Myzus persicae (Sulz.)[a]	100–200	90	76	84[b]
	5	55	10	18
Aphis gossypii Glover	50	10	9	90
	25	3	0	0
	5	10	1	10
Aphis medicaginis Koch	100–150	42	19	45
Aphis rumicis Linn.	100–200	18	14	80
	50–75	15	5	33
	20	2	0	0
Macrosiphum solanifolii (Ashm.)	125	8	2	25
Micromyzus formosanus (Tak.)	200	7	1	14
Amphorophora sonchi (Oest.)	150	6	0	0
Rhopalosiphum pseudobrassicae (Davis)	10	15	0	0
Rhopalosiphum (*Aphis*) *maidis* (Fitch)	50–100	26	0	0
Tetranychus sp., probably *althaeae* v. Hanst	300	18	0	0

[a] The number of tests listed for *Myzus persicae* does not include all of those conducted with the species but represents only those involving high and low numbers of individuals per test.

[b] Of the 14 negative tests, 13 occurred in an experiment involving old inoculum which was a poor virus source.

Some of the other species have been reported to breed on papaya in nature, however, and it is frequently true that insects which sometimes colonize on certain hosts in nature cannot readily be induced to breed by caging them on such plants immediately after removal from a preferred host.

As indicated in table 1, *Myzus persicae*, *Aphis gossypii* Glover, *A. medicaginis* Koch, and *A. rumicis* Linn. were clearly shown to be vectors of papaya ringspot. *Macrosiphum solanifolii* (Ashm.) with 2 out of 8 tests positive is apparently a vector although more confirmation is needed. *Mi-*

[3] The writer wishes to express his appreciation to Professor E. O. Essig of the University of California for his determination of the aphids used in these tests and to Mr. E. A. McGregor, of the U. S. Bureau of Entomology and Plant Quarantine, for identifying the red spiders.

cromyzus formosanus (Tak.) with one out of 7 tests positive may be capable of transmitting the virus, but should be tested more extensively. The number of tests listed for *M. persicae* does not represent all of the experiments involving this species, but includes only those in which either very high or very low numbers of individuals were used per test.

Myzus persicae. Among the insects which breed on papaya in Hawaii, *M. persicae*, the green peach aphid, is found more commonly on papaya than any other insect. Moreover, it greatly surpasses all other species in the density of its populations on papaya. The writer has found several thousand aphids on a few small sucker shoots growing on a single plant. The green peach aphid apparently has little if any toxic effect upon papaya since small plants four inches tall each carried several hundred aphids for a week without marked effect. However, the apical leaves of small, slow-growing sucker shoots produced on the main stem of field trees sometimes are slow to expand when the under surface carries a dense population of aphids. This retardation in growth is probably due to the quantity of plant juice withdrawn by the insects, and not to introduction of a toxin as appears to be the case with *Aphis rumicis.*

Myzus persicae feeds primarily in the veins and veinlets of the leaves. Normally the aphids select the under surface of the leaf but heavy populations frequently extend over the upper surface as well. The tender terminal stem of the young plant is also fed upon. When *M. persicae* was caged on papaya fruits, some of the aphids fed. The feeding punctures in green papaya fruits exuded latex.

The population of *Myzus persicae* in the field in Hawaii fluctuates with the season, weather conditions, and with the abundance of natural enemies. During the cool spring months of 1945 and 1946 the aphids were very abundant on papaya, particularly in the Kailua district. During the warmer, drier summer months the population declined to a relatively low level on papaya although a few heavy colonies could always be found.

The incidence of natural spread of ringspot disease in the papaya plots maintained in Manoa Valley paralleled the field population of the aphid.

In addition to papaya, *Myzus persicae* breeds on a wide range of other cultivated and weed hosts in Hawaii.

Since it is a demonstrated vector of papaya ringspot virus and frequently becomes very abundant on the papaya plant, *Myzus persicae* undoubtedly is responsible for most of the spread of ringspot disease in Hawaii. A few other species of aphids occasionally reproduce on papaya and winged forms of many species are casual visitors to this plant. Some of these species have been found experimentally to be vectors and they are prebably responsible for a limited amount of ringspot transmission in nature.

Aphis gossypii. The melon aphid has a wide host range and is encountered more commonly in Hawaii than any other aphid. Holdaway

(1) and Look and McAfee (5) have recorded it as breeding on papaya in Hawaii.

Aphis medicaginis. The bean aphid is common in Hawaii on legumes, *Portulaca oleracea* L., *Amaranthus* sp., and other hosts. Look and McAfee (5) state that it has been observed breeding in abundance on papaya.

The writer has occasionally found apterous *Aphis gossypii* and *A. medicaginis* on papaya in relatively large numbers. However, in each instance the aphid infestation could be traced to preferred breeding host plants which were growing near the papaya tree. In several such cases the recent drying up of the preferred hosts, due to drought or cultivation, had caused a migration of the aphids to nearby green plants including papaya. When caged on papaya the melon aphid and the bean aphid reproduce to a slight extent but the colonies die out within a week or 10 days. Both species were shown to transmit papaya ringspot virus.

Aphis rumicis. During most of the year this aphid is rarely encountered at the lower elevations on Oahu. It can usually be found, however, on fireweed, *Erechtites valerianaefolia* D. C., in the mountains. During March and April, 1946, it occurred abundantly on *Nothopanax guilfoylei* Merr., in Manoa Valley where the papaya ringspot tests were conducted. When caged on papaya, *A. rumicis* survived for 10 days, feeding primarily on the lower surface of the leaves. Approximately 90 percent of the aphids fed in the veins and veinlets of the leaves. Moulting and some reproduction occurred. This aphid produces a toxic effect on papaya in addition to transmitting ringspot virus. After 100 aphids had fed on test trees 18 inches tall for 2 days the 2 youngest partially expanded leaves began to droop and in 3 days they were limp. Experiments summarized in table 1 demonstrated the ability of *A. rumicis* to transmit ringspot virus.

Macrosiphum solanifolii. In Hawaii the potato aphid breeds most abundantly on potato and tomato but it is also reported to reproduce on several other plants including papaya (5). On papaya this aphid survived in cages for 10 days and a few produced progeny. Most of the aphids moved to the underside of the leaves where they fed primarily in the veins and veinlets. Alatae[4] usually survived longer on papaya than did the apterae.[4]

Only 8 ringspot tests were conducted with this aphid. Two of the 8 test trees developed positive though rather mild symptoms. More extensive tests should be carried out with this species.

Micromyzus formosanus. The onion aphid breeds only on onion and chives in Hawaii (5). It was used in 7 ringspot tests to determine whether the virus could be transmitted by species which are unrelated to the more common vectors and which find papaya unacceptable as a host. Transmission occurred in only one case. Although no natural spread occurred among the control plants the work should be confirmed with a longer series before concluding that the onion aphid is a vector of papaya ringspot.

[4] Alatae are aphids which are winged in the adult stage. Apterae are aphids which are wingless in the adult stage.

Amphorophora sonchi (*Oestlund*). This species breeds commonly only on *Sonchus oleraceus* in Hawaii. It did not feed well on papaya and failed to transmit the virus in the 6 tests in which it was used.

Rhopalosiphum pseudobrassicae (*Davis*) *and Rhopalosiphum* (*Aphis*) *maidis* (*Fitch*). Although these aphids fed readily on papaya for a short time they failed to transmit papaya ringspot in these experiments.

Tetranychus sp. probably *althaeae* v. Hanst. The red spider mites of this species are as a rule the most common and must numerous arthropods found on papaya plants in Hawaii. As indicated in table 1, the 18 attempts to transmit papaya ringspot virus with this species were unsuccessful.

VECTOR EFFICIENCY

The efficiency of aphid vectors in transmitting papaya ringspot virus varies considerably within a given species from time to time. Not all of the factors which affect transmission have been established experimentally, but the potency of the virus source is important and is discussed later.

In the tests involving *Myzus persicae,* the most important vector, the number of aphids used per test varied from one individual to several hundred. An indication of the efficiency with which this species transmits ringspot virus can be obtained from a comparison of experiments involving small and large numbers of aphids as presented in table 1. In tests with 5 aphids per plant, transmission was obtained to 10 out of 55 plants, or 18 per cent of the total. In 90 tests with 100 to 200 aphids per plant, 76 plants, or 84 per cent, developed symptoms and 14 failed to acquire the disease. Of the 14 negative tests, however, 13 occurred in an experiment involving old inoculum which proved to be a poor source of virus. As is evident in table 1, 100 or more aphids per plant will usually insure transmission of the disease, providing the aphids have had the opportunity of feeding on a plant carrying virus in relatively high concentration.

FEEDING PERIODS REQUIRED FOR ACQUIRING AND TRANSMITTING VIRUS

Papaya ringspot virus belongs in the group of viruses designated as non-persistent by Watson and Roberts (7) in 1939. Such viruses require no latent period in their insect vectors and are not retained long by their vectors after the latter leave diseased plants.

During this investigation, *Myzus persicae* was shown to acquire ringspot virus from a diseased plant in a feeding time as short as 2 minutes and to infect healthy plants in a feeding period of 5 minutes. Shorter feeding periods were not tested in adequate numbers to determine the minimum feeding time, designated "threshold" by Storey (6) in 1938, necessary to acquire the virus from a diseased plant or to infect a healthy plant.

In experiments summarized in table 2, winged *Myzus persicae* previously starved for 4 or 24 hours were allowed feeding periods of 2 or 5 minutes on diseased leaves. The aphids were then transferred immediately to healthy test plants where they fed for 5 minutes. They were then transferred to

a second and third series of healthy plants. As indicated in table 2, the aphids were able to acquire and transmit ringspot virus in a total time period as short as 7 minutes. Further investigation will probably show that even shorter feeding periods may also result in transmission. These data suggest that the virus has no measurable latent period in the aphid vector.

TABLE 2.—*Loss of papaya ringspot virus by infective Myzus persicae (Sulz.) on the first healthy plant fed upon in serial transfers and proof that aphid vectors may acquire and transmit ringspot virus during short feeding periods*

Number of tests	Number and stage of aphids per plant (1st series)	Fasting period of aphids before acquisition feeding period	Acquisition feeding time on inoculum	Feeding time on 1st test plant	Results		Number of aphids to each 2nd healthy plant	Feeding time on 2nd test plant	Results	
					+	−			+	−
6	200 apterae	0	24 hr.	4 hr.	6	0	50 apterae[a]	2 days	0	6
5	25 apterae	0	18 hr.	2 days	4	1	10 apterae	2 days	0	5
5	25 apterae	0	18 hr.	2 hr.	4	1	25 apterae	2 days	0	5
7	15 alatae	4 hr.	25 min.	2 hr.	1	6	14 alatae[a]	2 days	0	7
9	15 alatae	24 hr.	5 min.	5 min.	5	4	15 alatae	5 min.[a]	0	9
1	15 alatae	4 hr.	2 min.	5 min.	1	0	15 alatae	2 days	0	1
1	10 alatae	4 hr.	2 min.	2 min.	0	1	10 alatae	2 days	0	1
34					21	13			0	34

[a] Aphids in these tests were allowed a 5-minute feeding period on the second series of plants and were then transferred to a third series where they fed for 2 days. No transmission occurred in the second or third series.

VIRUS RETENTION EXPERIMENTS

Several experiments were conducted in the greenhouse to determine the length of time *Myzus persicae* retains papaya ringspot virus after being transferred from a diseased plant to non-infected hosts.

Large colonies of aphids were transferred from ringspot papaya plants to potato plants upon which they were allowed to feed for periods of time varying from 3 to 21 days. Such aphids transferred from the potato plants to healthy papayas invariably failed to carry ringspot virus.

In a second experiment thousands of aphids, reared on pakchoi, were transferred to ringspot papaya foliage where they were allowed to feed for 24 hours. Aphid-infested ringspot foliage was then cut from the infected plant and laid on pakchoi plants. As the papaya foliage wilted, over a period of 18 hours, the infective aphids crawled to the pakchoi plants where feeding was resumed. Aphid-infested pakchoi leaves were then detached and caged on 15 healthy papaya plants. During the following 12 hours most of the aphids moved to the papaya foliage where they fed. At least 500 aphids fed on each test plant. No infection resulted in any of the papayas used in the experiment.

In a third experiment (Table 2) 200 infective *Myzus persicae* were transferred by brush to each of 6 healthy papaya plants. After feeding for 4 hours on the test plants 50 apterae were transferred from each of the 6 test plants to a second healthy papaya. All of the first 6 plants developed the disease while none of the plants in the second series of 6 became infected.

Table 2 summarizes the results obtained in several additional experiments involving serial transfers of aphids to two or more healthy plants. In some of these tests aphids were forced to fast for a period of 4 or 24 hours prior to feeding on inoculum. In most of such tests the feeding periods for acquiring and transmitting the virus were shortened to 2 or 5 minutes. The results in table 2 demonstrate that in serial transfers, with the transmission feeding time as short as 5 minues per plant, *Myzus persicae* loses the virus on the first healthy plant fed upon. However, if more extensive tests were run with the feeding time of previously fasted aphids reduced to an even shorter period it is probable, in view of the results obtained by Watson and Roberts (8) with other non-persistent viruses, that *M. persicae* could be shown, in a few instances, to transmit the virus to the second or third plant fed upon. Under field conditions in Hawaii, however, it is probable that aphid vectors rarely if ever retain the virus long enough to infect more than a single papaya plant after leaving a ringspot tree.

EFFECT OF VIRUS SOURCE ON INSECT TRANSMISSION

The length of time a papaya plant has been infected with ringspot virus has an effect either upon the concentration of the virus in the plant or upon the availability of the virus to *Myzus persicae*.

Table 3 presents the results of an experiment dealing with this phe-

TABLE 3.—*Influence of virus source on papaya ringspot transmission by Myzus persicae. Approximately 150–200 aphids per test, transferred on pieces of diseased leaves*[a]

Type of inoculum[b]	Number of tests	Positive	Negative	Percentage transmission
Old	7	3	4	43
Old	10	9	1	90
Old	11	3	8	27
Totals for old inoculum	28	15	13	53.6
Young and old	1	1	0	100
Young and old	17	17	0	100
Young and old	6	6	0	100
Young	2	2	0	100
Young	3	3	0	100
Totals for tests including young inoculum	29	29	0	100

[a] Sixty check trees remained disease free.

[b] Old inoculum = young leaves from papaya plants which had been diseased for three months. Young inoculum = young leaves from papaya plants on which symptoms had been evident only 10 days prior to use in this experiment.

nomenon. The insects used in this experiment came from the same stock. They were reared in the greenhouse on pakchoi. The test plants used were 3 to 4 feet in height and were growing under the same field conditions. Although the test feedings took place on leaves of varying maturity among the test plants, both groups included plants inoculated in old and young leaves. Moreover, the data indicate that the age of the leaf into which the aphids inoculated the virus was not a limiting factor. The only apparent effective variable was the length of time the inoculum trees had been diseased.

Inoculum recorded as old was composed of young leaves from plants on which symptoms had been evident for three months prior to use in this experiment. Young inoculum came from plants which had had symptoms for only ten days.

As is evident in table 3, *Myzus persicae* (150–200 aphids per plant) infected 100 per cent of the plants fed upon in all cases in which some or all of the aphids had fed upon young inoculum. In contrast, only 53 per cent of the plants were infected in tests in which only old inoculum had been available to the aphid vectors. Moreover, the results suggest there was considerable variability in the old inoculum from different plants or of different leaves on the same plant. In one series, with old inoculum, 9 of the 10 plants were infected. In another series only 3 out of 8 plants were infected.

FAILURE TO TRANSMIT VIRUS FROM DISEASED FRUITS

Papaya fruits are transported freely in commerce and privately from place to place on the island of Oahu and to a lesser extent between the various islands of the Hawaiian chain. The possibility of infected fruits serving as an adequate virus source for aphid vectors in sections of Oahu, and on islands where papaya ringspot is not known to occur, constituted a potential disease hazard which, if confirmed, might result in a quarantine against papaya fruit movement in the Hawaiian islands.

Under natural conditions *Myzus persicae* feeds primarily on the leaves of the plant. The petioles and main stem of young, tender plants are also attractive feeding sites. In no instance have aphids been found colonized or feeding on fruit in the field. Nevertheless it is possible that occasional feeding on the fruit occurs.

To test the possibility of obtaining transmission from ringspot fruits by means of aphids, large numbers of *Myzus persicae* were confined on infected fruits ranging in maturity from young (one inch in length) to ripe yellow. The aphids were caged on the fruits for from 1 to 2 days. Only those individuals actually observed feeding on the fruits were transferred to the foliage of test plants. Each plant received either 25 or 50 aphids. No transmission was obtained from fruits in 21 tests conducted. These results suggest that aphids rarely, if ever, transmit ringspot virus from infected fruits.

SUMMARY

Myzus persicae (Sulzer), the most common insect on papaya in Hawaii, is the major insect vector of ringspot virus. *Aphis gossypii* Glover, *Aphis medicaginis* Koch, and *Aphis rumicis* Linn. are also shown to be vectors of the disease. *Macrosiphum solanifolii* (Ashm.) and *Micromyzus formosanus* (Tak.) are probably also vectors but the test series with these species were too few to be conclusive.

Myzus persicae acquired the virus in a feeding time of 2 minutes and infected healthy plants in a feeding period of 5 minutes.

There is no demonstrable latent period of the virus in the aphid vector and the virus is not retained by the vector beyond the first healthy plant fed upon after transfer from a diseased plant.

Groups of 150–200 *Myzus persicae* induced infection in 100 per cent of the test plants after transfer from trees showing ringspot symptoms for only 10 days. Similar numbers of aphids transmitted the disease to only 53 per cent of the test plants after transfer from trees which had been diseased for 3 months.

Myzus persicae failed to transmit ringspot virus from infected fruits.

UNIVERSITY OF HAWAII AGRICULTURAL EXPERIMENT STATION
HONOLULU, T. H.
AND
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. HOLDAWAY, F. G. Insect pests of papaya and their control. *In:* Papaya production in the Hawaiian Islands. Univ. Haw. Agr. Exp. Sta. Bul. 87: 45–51. 1941.
2. JENSEN, D. D. Virus diseases of plants and their insect vectors with special reference to Hawaii. Proc. Haw. Ent. Soc. 12: 535–610. 1946.
3. ———. A new virus disease of papaya. Univ. Haw. Agr. Exp. Sta. Biennial Rept. 1944–1946: 67. 1947.
4. ———. Papaya virus diseases with special reference to papaya ringspot. Phytopath. 39: 191–211. 1949.
5. LOOK, WM. C., and W. L. MCAFEE. New host records of aphids in Hawaii. Proc. Haw. Ent. Soc. 12: 99–112. 1944.
6. STOREY, H. H. Investigations of the mechanism of the transmission of plant viruses by insect vectors. II. The part played by puncture in transmission. Proc. Roy. Soc. London, B. 125: 455–477. 1938.
7. WATSON, M. A., and F. M. ROBERTS. A comparative study of the transmission of *Hyoscyamus* virus 3, potato virus Y, and cucumber virus 1 by the vectors *Myzus persicae* (Sulz.), *M. circumflexus* (Buckton), and *Macrosiphum gei* (Koch). Proc. Roy. Soc. London, B. 127: 543–576. 1939.
8. ——— and ———. Evidence against the hypothesis that certain plant viruses are transmitted mechanically by aphids. Ann. Appl. Biol. 27: 227–233. 1940.

SOME RELATIONS BETWEEN PHASEOLUS VIRUS 2 AND ITS ASSOCIATED CRYSTALLINE INCLUSIONS

SAUL RICH[1]

(Accepted for publication November 18, 1948)

McWhorter (4) found crystalline inclusions in the nucleoli and cytoplasm of plants infected with Phaseolus virus 2. These inclusions were not found in virus-free specimens. The trypan blue staining technique, first described by McWhorter (3) and later modified by Rich (6), made the detection and observation of these crystals a simple matter. The chemical nature and probable origin of these inclusions have also been described (7). The following paper describes further points of correlation between Phaseolus virus 2 and its associated crystalline inclusions.

SOURCE OF VIRUS

The virus studied was obtained from Blue Lake beans growing at Grand Island, Oregon. The virus proved to be unmixed and produced uniform symptoms through repeated subinoculations.

METHODS

All plant inoculations were made by the carborundum technique (5). The source plant material was ground in a sterile mortar and pestle, and then strained through cheesecloth. The resultant plant extract was applied to the healthy plant leaves with a cotton swab held in sterile forceps. Carborundum powder (600-mesh) was dusted on the leaves to be inoculated prior to swabbing.

All cytological observations were made on unfixed tissues stained with trypan blue.

RELATION BETWEEN THE TIME OF SYMPTOM APPEARANCE AND THE PRODUCTION OF CRYSTALLINE INCLUSIONS

Working with severe etch virus on tobacco, Sheffield (8) found the following results: " . . . rubbed leaves usually showed symptoms within five days, and on the sixth day the veins of younger leaves became cleared. At this time no abnormalities may be seen in either the nuclei or the cytoplasm. On the following day as many as a dozen crystals may be counted in many of the nuclei."

In the present study, broad bean plants (*Vicia faba* L.) inoculated with Phaseolus virus 2 were examined daily from the time of inoculation until after the appearance of symptoms. No crystals were found until the seventh day following the inoculation, before external symptoms were evident. The few small crystals found were only in nucleoli, and stained

[1] Now with the Department of Plant Pathology, Connecticut Agricultural Experiment Station, New Haven, Connecticut.

weakly with trypan blue. In the next two days, vein clearing became evident and the nucleolar inclusions became much larger, more abundant, and more easily stained. No cytoplasmic inclusions were present, but they appeared later. Cytoplasmic inclusions were produced earliest and in the greatest abundance in young plants which were growing vigorously at the time of inoculation.

To summarize, with Phaseolus virus 2 on broad bean, no crystals were found until a day or two before the first appearance of symptoms, i.e., vein clearing of the youngest leaflets. This observation differs from Sheffield's report in that severe etch inclusions did not appear until two days following the production of symptoms.

RELATION BETWEEN EXTERNAL SYMPTOM PRESENCE AND THE PRESENCE OF CRYSTALLINE INCLUSIONS

It has been found in the case of certain systemic plant viruses that leaves that are mature before the appearance of symptoms remain externally

TABLE 1.—*Distribution of inclusions in broad bean plants just beginning to show external symptoms of Phaseolus virus 2*

Plant part	External symptoms	Inclusions
Stem growing point	Absent	Absent
Young leaves	Absent	Present
Young leaves	Present	Present
Mature leaves near growing point	Absent	Present
Lowest mature leaves	Absent	Absent
Stem internodes[a]	Absent	Present
Roots	Absent	Absent

[a] Inclusions were also present in the stem between ground level and the node of the lowest mature leaf.

symptomless (1, p. 86). This is also true of Phaseolus virus 2 on broad bean. For this study, plants were chosen which had some leaves with well-defined symptoms and some without external symptoms. All leaves on these plants were examined. Those externally symptomless leaves nearest the growing point contained well-developed crystalline inclusions; leaves nearest the ground level were not only externally symptomless, but also were devoid of any visible inclusions. Inclusions were always present in leaves with symptoms.

Fulton (2) reported the presence of virus inclusions in the roots of tobacco plants infected with tobacco mosaic. He could find no definite external symptoms of disease on infected roots.

Broad bean plants having well-developed symptoms of Phaseolus virus 2 on their aerial portions were examined repeatedly for crystalline inclusions in their roots. No inclusions were present in roots of infected plants.

Table 1 summarizes the relation between external and internal symptoms of Phaseolus virus 2.

Inclusions were absent from the root tissues, the growing points of stems, and the oldest leaves. Small crystals were present in the nucleoli of the youngest leaves. The stem, down to the ground level, contained inclusions. Crystals were present in the stem even in the internodes between leaves in which no inclusions were found.

These observations indicate that crystalline inclusions may be induced by Phaseolus virus 2 in parts of broad bean plants without the production of external symptoms.

RELATION BETWEEN PRESENCE OF VIRUS AND PRESENCE OF CRYSTALLINE INCLUSIONS

The following results (Table 2) demonstrate the relation between the presence of recoverable virus and the presence of crystalline inclusions. The inocula were selected from previously examined portions of infected plants. As only five plants were inoculated in each case, additional data are necessary in order to show whether there is no virus in the roots, or whether the root extract decreased the infectivity of the virus. Fulton (2) found that tobacco mosaic virus from the roots of host plants was actually less virulent and less resistant to thermal inactivation than was the virus contained in the aerial portions of the plants.

TABLE 2.—*Relationship between presence of virus and presence of inclusions*

Inoculum used			Results of inoculation
Part of plant	Symptoms	Inclusions	
Leaf	Present	Present	Positive
Leaf	Absent	Present	Positive
Leaf	Absent	Absent	Positive
Root	Absent	Absent	Negative

The data presented in table 2 demonstrate that infective virus may be present regardless of whether or not the plant part has either external symptoms or internal inclusions. Crystalline inclusions, however, were found only when the virus was present.

SUMMARY

In broad beans newly infected with Phaseolus virus 2, crystalline inclusions were produced just prior to the first appearance of external symptoms.

Broad bean plants infected with Phaseolus virus 2 may have parts in which no external symptoms appear and yet crystalline inclusions are present.

Virus-infected broad bean plants may have portions that have neither external symptoms nor crystalline inclusions, and yet infective quantities of virus may be obtained therefrom. Crystalline inclusions were found only in plant parts that contained infective quantities of virus.

Broad beans infected with Phaseolus virus 2 showed no root symptoms, nor were crystalline inclusions found in the roots. Virus infection could not be obtained when roots of infected plants were used as inoculum.

OREGON STATE COLLEGE
CORVALLIS, OREGON

LITERATURE CITED

1. COOK, M. T. Viruses and Virus Diseases of Plants. 244 pp. Burgess Publishing Co., Minneapolis, Minn. 1947.
2. FULTON, R. W. The behavior of certain viruses in plant roots. Phytopath. 31: 575–598. 1941.
3. MCWHORTER, F. P. Separation of Tulip 1 virus from Lily-latent by cytological methods. Phytopath. 30: 788. 1940.
4. ———. Isometric crystals produced by Pisum virus 2 and Phaseolus virus 2. Phytopath. 31: 760–761. 1941.
5. RAWLINS, T. E., and C. M. TOMPKINS. The use of carborundum as an abrasive in plant-virus inoculations. Phytopath. 24: 1147. 1934.
6. RICH, S. A rapid stain method for detecting certain plant virus inclusions. Stain Technology 23: 19–20. 1948.
7. ———. The chemical nature and origin of *Phaseolus* virus 2 crystalline inclusions. Science 107: 194. 1948.
8. SHEFFIELD, F. M. L. Virus diseases and intracellular inclusions in plants. Nature 131: 325–326. 1933.

THE VIRUS OF TOBACCO LEAF-CURL

D. G. SHARP[1] AND F. A. WOLF[2]

(Accepted for publication November 13, 1948)

Tobacco leaf-curl or kroepoek is one of the most serious diseases that affects tobacco and certain other kinds of plants in Venezuela (11). The causal agent of this disease is a virus, and several species of white-flies (Aleyrodidae) have been found to serve as its vectors. Recently, an opportunity was presented for studies on some of the physical characteristics of the leaf-curl virus itself. The virus has been examined in crude sap by means of the electron microscope and a similar investigation has been made on the agent concentrated and partially purified by ultracentrifugation. In addition, some of the sedimentation characteristics of the agent have been determined. The results of these studies and a comparison of the properties of the leaf-curl virus with those of other viruses causing disease in the tobacco plant (1, 2, 3, 6, 7, 8, 9, 13) are reported in the present paper.

GENERAL EXPERIMENTAL PROCEDURES

Although plants affected with leaf-curl were abundantly available in Venezuela, equipment for the contemplated study was not available there. Moreover, importation into the United States of living diseased plants is prohibited. For these reasons the present study was necessarily limited to the use of formolized sap from diseased plants that was sent to the United States. Young leaves of leaf-curl affected plants growing in a field at hacienda Paya, situated near Turmero, in the State of Aragua, were collected and taken to the laboratory of the Departamento de Fitopatología in Maracay. As soon as possible thereafter, the leaves were macerated by passage through a meat grinder. The sap was then pressed out by hand and passed through a coarse cloth that retained only the larger leaf fragments. Formaldehyde was added, as a preservative, in a concentration of 0.1 per cent. The formolized sap was then sent by air-express to the virus laboratories of the Department of Surgery, in the School of Medicine, at Duke University. Here, the material was stored in a refrigerator maintained at 2° to 6° C.

Some of the studies were made on the crude sap. After the crude suspension had stood in the refrigerator for a few days, much of the suspended plant tissue had sedimented leaving a turbid slightly greenish supernatant. Collodion screens for electron micrography of the virus in the sap were

[1] Department of Surgery, School of Medicine, and [2] Department of Botany, Duke University, Durham, North Carolina.

This study was made possible by the generous help of Dr. Clifford H. Meredith, Jefe, Departamento de Fitopatología, Maracay, Venezuela. One of us was employed during the past year by the Ministero de Agricultura y Cría, in cooperation with the Compañia Anónima Cigarrera Bigott, Sucs., and the Compañia Anónima Venezolana de Tabaco. The encouragement and assistance given by these agencies is greatly appreciated.

prepared in the usual way, using samples of this supernatant removed with a pipette. The preparations were shadowed with gold. The screens were set at an angle of 11 degrees to the source of evaporating gold or in such a position that the virus particles cast shadows about five times as long as particle height (10).

Concentration and partial purification of the virus were effected in an air-driven quantity ultracentrifuge. A volume of 30 ml. of the supernatant of the sap suspension was spun in an angle centrifuge for 15 minutes at 3,000 g. to remove aggregated material. The nearly clear greenish supernatant was distributed in 2 ultracentrifuge tubes and spun in the air-driven machine for 60 minutes at 25,000 g.

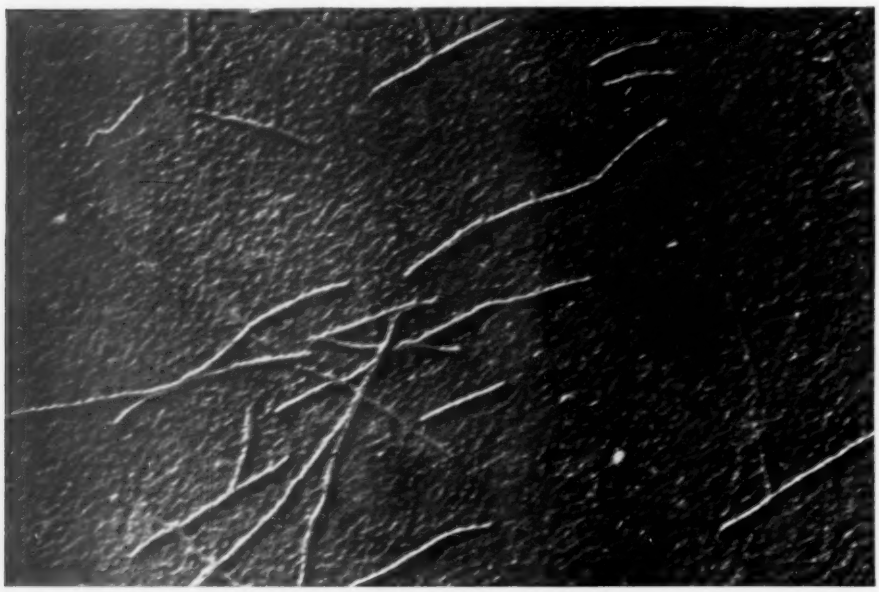

FIG. 1. Electron micrograph of formolized but undiluted crude sap of tobacco plant affected with leaf-curl showing rod-like virus particles. Gold shadow cast at 11 degree angle. Magnification 40,000 ×.

The slightly greenish pellets were resuspended in 15 ml. of 0.1 M phosphate buffer of pH 7.0. This suspension was then centrifuged at 3,000 g. for 15 minutes, and the sediment was discarded. The supernatant fluid was transferred to another tube and was again spun at 25,000 g. for 60 minutes. The large clear pellet that formed was resuspended in 2 ml. of the phosphate buffer, and this suspension is herein regarded as the virus concentrate. Portions of this suspension were prepared for election microscopy as described in the instance of the crude sap.

ELECTRON MICROSCOPY

A representative photograph of the crude sap showing rod-like particles is given in figure 1. The number of these rod-like virus particles in such

preparations is seen to be small and, although their length varies, their width is rather uniform. Several such preparations were photographed, and the lengths of 67 sharply delimited particles were measured. These measurements range in length up to 1144 mμ. with a strong maximum at 277 mμ. The results of the measurements, in histogram form, appear in figure 3, A.

The virus concentrate to be examined was diluted with an equal volume of distilled water. A representative micrograph of the virus shadowed with gold showing large numbers of the rod-like virus is given in figure 2. The distribution of lengths from measurement of 200 particles, presented in

FIG. 2. Electron micrograph of formolized ultracentrifugally concentrated sap of tobacco plant affected with leaf-curl. Gold shadow cast at 11 degree angle. Magnification 40,000 ×.

histogram form in figure 3, B, shows a strong maximum at 285 mμ. The width is exceedingly uniform and, when estimated from rods that lie side by side, is about 15 mμ.

SEDIMENTATION VELOCITY STUDIES

The sedimentation velocity of the concentrated virus was measured in an air-driven vacuum type ultracentrifuge carrying a cell 5 mm. thick and 12 mm. high, at a mean turning radius of 6.5 cm. In the first run, the series of pictures (Fig. 4, A) shows the particles of the concentrate sedimenting as a single exceedingly sharp boundary. Other runs were made on this material diluted 1 : 2, 1 : 4, and 1 : 8 with 0.1 M phosphate buffer of pH 7.0. As the concentration decreased the boundary became progressively more diffuse. Figure 4, B shows the boundary obtained with the 1 : 8 dilution. In some of the diluted samples a faint secondary boundary could be seen

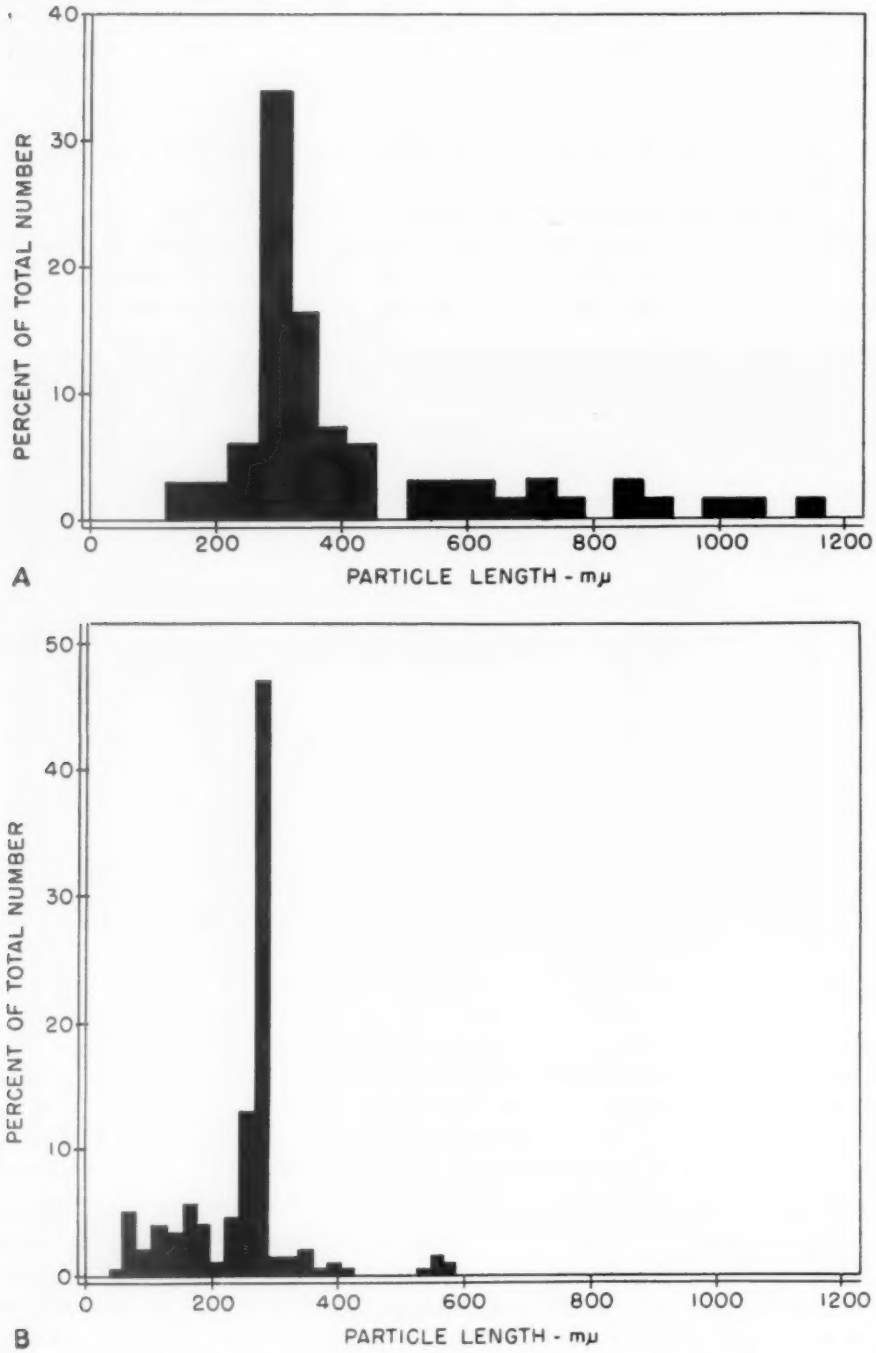

FIG. 3. A. Particle length distribution as seen in electron micrographs of crude material. B. Particle length distribution as seen in ultracentrifugally purified material.

sedimenting faster than the principal one. The sedimentation constants calculated from these four samples in order of dilution were $Sw_{20} = 169$, 176, 170, and 171×10^{-13} c.g.s. units.[3] The faster boundary, seen best in the second (1:2 dilution) run, gave $Sw_{20} = 211 \times 10^{-13}$. Although dilution rendered the boundary more diffuse, it did not change the sedimentation rate as is known to occur for many purified viruses (4).

FIG. 4. Sedimentation diagrams of the formalin-treated virus of tobacco leaf curl. a. The concentration is 15 × that in the crude sap and the sedimentation constant calculated from this series of 5-minute-interval pictures at 15,150 R.P.M., is $Sw_{20}o = 169 \times 10^{-13}$. b. The same material diluted 1:8. The time interval and speed are 3 minutes and 20,250 R.P.M. respectively, and the sedimentation constant for the principal boundary below it is $Sw_{20}o = 211 \times 10^{-13}$.

DISCUSSION

The most striking feature found from examination of the formolized sap from leaf-curl-affected tobacco plants is the morphologic similarity of the rod-shaped particles to the virus of ordinary tobacco mosaic. The images of the rods shown in the micrographs herein are identical in appearance with the rods of tobacco mosaic virus in the micrographs of Sigurgeirsson and Stanley (8); moreover, the histograms of length distribution of the two viruses closely resemble each other. The abundance of virus particles in the crude sap of leaf-curl-affected plants is also like that of ordinary mosaic. These similarities are the more remarkable when it is borne in mind that the clinical aspects of tobacco leaf-curl and ordinary mosaic are so different that no one could possibly confuse the two diseases.

Further similarities between the virus of tobacco leaf-curl and that of ordinary mosaic are seen in the sedimentation constants. It is known that under identical laboratory conditions all strains of mosaic virus do not give the same sedimentation constant. The values reported herein for the tobacco leaf-curl virus fall within the range of those in the literature for mosaic virus (13). Even the secondary boundary is like that seen by Wyckoff (12), using three different preparations of tobacco mosaic virus. In one respect only, and that perhaps a minor one, do the sedimentation characteristics of the leaf-curl virus differ from those of the mosaic virus. It is well established that when suspensions of purified viruses are successively diluted the sedimentation rate increases significantly although slowly (4). The range of concentrations used here is sufficient to show such an effect with the leaf-curl virus had it existed to the extent that it does with other viruses.

[3] Sw_{20} is the sedimentation constant reduced to the viscosity of water at 20° C. and expressed in units of centimeters, grams, and seconds (c.g.s.)

It is fully appreciated that biologically active materials may be morphologically indistinguishable yet may induce very dissimilar reactions or responses. Speculation at this time on the underlying reasons for this situation in the case of viruses is quite pointless and meaningless, at least until more is known about the composition and structural configuration of virus proteins.

SUMMARY

The formalin-treated sap of tobacco plants affected with leaf-curl has been studied with the electron microscope. After concentration and partial purification with the ultracentrifuge, further studies were made with the electron microscope, as well as with the analytical ultracentrifuge. All preparations were found to contain rod-like particles, that were regarded as the leaf-curl virus. These particles range in length up to 1140 mμ., but most of them are approximately 280 mμ. long. Their width is uniformly about 15 mμ.

The average sedimentation rate of tobacco leaf-curl virus approximates 171×10^{-13}, which is within the range of variation in rate for ordinary tobacco mosaic virus.

The tobacco leaf-curl virus closely resembles in appearance and in rate of sedimentation certain other viruses that affect tobacco.

DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA

LITERATURE CITED

1. KNIGHT, C. A., and G. OSTER. The size of the particles of some strains of tobacco mosaic virus as shown by the electron microscope. Arch. Biochem. **15**: 289–294. 1947.
2. LAUFFER, MAX A. Ultracentrifugation studies on tobacco mosaic and bushy stunt viruses. Jour. Phys. Chem. **44**: 137–146. 1940.
3. ———. The size and shape of tobacco mosaic virus particles. Jour. Amer. Chem. Soc. **66**: 1188–1194. 1944.
4. ———. The influence of concentration upon the sedimentation rate of tobacco mosaic virus. Jour. Amer. Chem. Soc. **66**: 1195–1201. 1944.
5. MARKHAM, R., K. M. SMITH, and R. W. G. WYCKOFF. Electron microscopy of tobacco necrosis virus crystals. Nature (London) **159**: 574. 1947.
6. OSTER, G., C. A. KNIGHT, and W. M. STANLEY. Electron microscope studies of Dahlem, Rothamsted, and Princeton samples of tobacco mosaic virus. Arch. Biochem. **15**: 279–288. 1947.
7. STANLEY, W. M., and T. F. ANDERSON. A study of purified viruses with the electron microscope. Jour. Biol. Chem. **139**: 325–338. 1941.
8. SIGURGEIRSSON, T., and W. M. STANLEY. Electron microscope studies on tobacco mosaic virus. Phytopath. **37**: 26–38. 1947.
9. TAKAHASHI, W. N., and T. E. RAWLINS. An electron microscope study of tobacco mosaic virus extracted from pulp and juice after various periods of infection. Phytopath. **38**: 279–282. 1948.
10. WILLIAMS, R. C., and R. W. G. WYCKOFF. The thickness of electron microscope objects. Jour. Appl. Physics **15**: 712–716. 1944.
11. WOLF, F. A., W. H. WHITCOMB, and W. C. MOONEY. Leaf-curl of tobacco in Venezuela. (In manuscript.)
12. WYCKOFF, R. W. G. An ultracentrifugal study of the pH stability of tobacco mosaic virus protein. Jour. Biol. Chem. **122**: 239–247. 1939.
13. ———, J. BISCOE, and W. M. STANLEY. An ultracentrifugal analysis of the crystalline virus proteins from plants diseased with different strains of tobacco mosaic virus. Jour. Biol. Chem. **117**: 57–71. 1937.

VIRUS CONCENTRATION IN PLANTS ACQUIRING TOLERANCE TO TOBACCO STREAK

ROBERT W. FULTON

(Accepted for publication January 4, 1949)

A striking characteristic of the tobacco streak disease, particularly in the field, is the healthy appearance of the leaves which are formed after the development of systemic necrotic symptoms. Johnson (7) has shown that in this respect streak is similar to the ringspot type diseases from which the infected plants have been described by Price (10, 11) as recovering, or acquiring an immunity.

As pointed out by Johnson (7), the tobacco streak disease lends itself to a study of this phenomenon. The differences are distinct between the necrotically affected leaves and the subsequently developed non-necrotic leaves. These non-necrotic leaves, while slightly stunted on greenhouse plants, usually appear healthy in the field. "Skip leaves," or the appearance of necrosis on new leaves above symptomless leaves, as described for tobacco ringspot by McKinney and Clayton (9), do not occur, or occur only rarely on sucker growth.

In a previous publication (6) data were presented which indicated that in infected tobacco both those leaves having systemic necrotic symptoms and the upper, non-necrotic leaves contained a very low concentration of tobacco streak virus when compared with leaves which had been inoculated directly. Price (11) and later Stanley (14) reported that the tobacco ringspot virus was present in considerably higher concentrations in the necrotic leaves as compared to the non-necrotic leaves of infected plants. Since the tobacco streak virus apparently differed from tobacco ringspot virus in the relative concentrations in different tissues, it appeared to merit further investigation.

The presence of an inactivator in extracts from diseased plants also has been investigated, first because of a possible bearing on the recovery phenomenon, and second because prevention of inactivation was necessary for quantitative comparisons of virus concentration.

MATERIALS AND METHODS

Experimental plants were grown in composted soil in 4-inch pots at greenhouse temperatures of 75° to 80° F. For most of the work, the tobacco variety Wisconsin Havana 38 was used. Some comparisons were made using the Turkish variety Xanthia. The tobacco plants were inoculated after 4 to 6 leaves had developed. Samples for assay were taken after 3 or 4 symptomless leaves had developed above the systemically necrotic leaves.

The tobacco streak virus was a type commonly found in the field. The strain used was obtained from one local lesion on Great Northern bean inoculated with a field collection of virus. Since other strains have been isolated which differ in severity of symptoms produced, it seemed advisable to confine the work to a single strain.

Estimates of tobacco streak virus concentration were made by counting local lesions appearing on inoculated leaves of guar (*Cyamopsis tetragonalobus* (L.) Taub.). In comparing virus concentrations, usually 3 different preparations of inoculum from one source were applied to the left or right halves of 9 leaves on 3 guar plants. The comparable preparations from the other source were inoculated to the opposite half-leaves. Whenever possible, the preparations were arranged in a randomized latin-square design on the assay plants. When it was necessary to make comparisons between 3 or more sources or treatments, these were either paired on opposite half-leaves, or one treatment was selected as a standard and the others compared with it on opposite half-leaves. All inoculations were made on leaves lightly dusted with carborundum powder.

Because previous work (6) had indicated a rapid loss of infectivity in the expressed juice of streak-infected tobacco leaves, inoculations were made as rapidly as possible after grinding the tissue. The common method of making serial dilutions of expressed juice could not be used, because even the short interval between grinding the tissue and the final inoculation resulted in considerable loss of infectivity. Rather than serial dilutions, therefore, inocula containing different proportions of tissue were used. Disks were cut from lamina of infected leaves with a sterile 5/16-inch cork borer. The fresh weight of 100 such disks was about 1 gram. In preparing inocula, each disk was considered equivalent to 0.01 ml. of expressed juice. Ten disks, ground in 0.4 ml. of phosphate buffer at pH 7.0, are referred to as a tissue concentration of 1:5; and 10 disks in 2.4 ml. as a tissue concentration of 1:25. For the more dilute inocula, the tissue was ground in a small amount of buffer and the remainder pipetted into the mortar after grinding. Comparisons were thus made between equal areas of infected leaf tissues rather than between equal volumes of expressed sap.

In assaying the concentration of the tobacco ringspot virus, the inoculum was prepared in the same manner as with the tobacco streak virus. In most cases, the lesions produced on primary leaves of young cowpea plants were counted. When it was found that the tobacco ringspot virus also produced distinctive local lesions on guar, this plant was used in some trials.

In this paper, the term "acquired tolerance" is used to refer to the development of healthy appearing leaves which follow the appearance of necrotic symptoms of tobacco streak. For convenience, these upper, non-necrotic leaves of infected plants are referred to as "tolerant leaves," and leaves with systemic necrotic symptoms are referred to as "necrotic leaves."

EXPERIMENTAL RESULTS

The Presence of an Inactivator in Leaf Extracts

In preliminary comparisons of virus concentration in necrotic and tolerant leaves, it was found that dilution resulted in an increase in infectivity. The infectivities of four concentrations of necrotic tobacco leaf tissue, 1:1,

1:5, 1:25, and 1:125, were compared on opposite half-leaves of guar with the same concentrations of tolerant leaf tissue. In three trials, the average numbers of lesions obtained with tissue from necrotic leaves, in decreasing order of concentration, were 60, 96, 110, and 41. The figures for comparable inocula from tolerant leaves, in the same order, were 29, 61, 163, and 105. Only a small number of lesions was produced by the most concentrated inoculum, while the greatest infectivity was obtained with tissue concentrations of 1:25.

The increase in infectivity of the preparations with increasing dilution is similar to the reactivation of inactive virus in mixtures with certain inactivators, as has been described by Kuntz and Walker (8). The results suggested that the infected tissue contained some inactivator, and it seemed possible that such an inactivator might be related to the recovery phenomenon.

TABLE 1.—*The effect of extracts of healthy tobacco leaf tissue on the infectivity of a 1:25 tissue concentration of the tobacco streak virus. Treated inocula contained 20 per cent healthy leaf tissue*

Method of preparing inoculum	Total lesions on 6 half-leaves of guar in each of 5 trials				
	I	II	III	IV	V
Healthy tissue ground with infected tissue and buffer immediately before inoculation	306	182	66	491	766
Healthy tissue ground in buffer 20 minutes before grinding with infected tissue and inoculating	7	5	8	84	8
No healthy tissue added	453	327	112	631	712

Diachun (5) has shown that extracts of healthy tobacco leaves will inactivate the tobacco streak virus. Preliminary attempts to confirm his results were occasionally unsuccessful. The variations were found to be due to differences in the time that the healthy extracts were exposed to air before mixing with virus. In one series of trials, inoculum was prepared containing 1:25 tissue concentration of tobacco streak virus and 20 per cent healthy tobacco leaf tissue. The effect of grinding both tissues together and inoculating immediately was compared with the effect obtained when the healthy leaf tissue was ground 20 minutes before grinding the infected tissue and inoculating. The results of 5 trials are presented in table 1. Healthy tissue, in most trials, had a slight inactivating effect. After being ground and exposed to air for 20 minutes, the inactivating effect was pronounced.

It seemed likely that the reactivating effect obtained by diluting infected tissue might be related also to the formation of an inactivator in the crushed infected tissue. To test this possibility, inocula from a single infected tobacco leaf were prepared in 3 concentrations, 1:1, 1:5, and 1:25.

Comparisons of the infectivities of the preparations were made immediately after grinding, and after 5, 10, and 20 minutes. The results of one trial are shown in figure 1, and were typical of other trials. The infectivity of the 1:1 preparation decreased very rapidly; that of the 1:5 preparation somewhat less rapidly. The 1:25 preparation lost no infectivity within 20 minutes. At the end of 20 minutes, the 1:25 preparation was the most infectious and the 1:1 preparation the least infectious.

It thus appeared that the low infectivity of the higher concentrations of inoculum in the preliminary trials was due to the formation of an inacti-

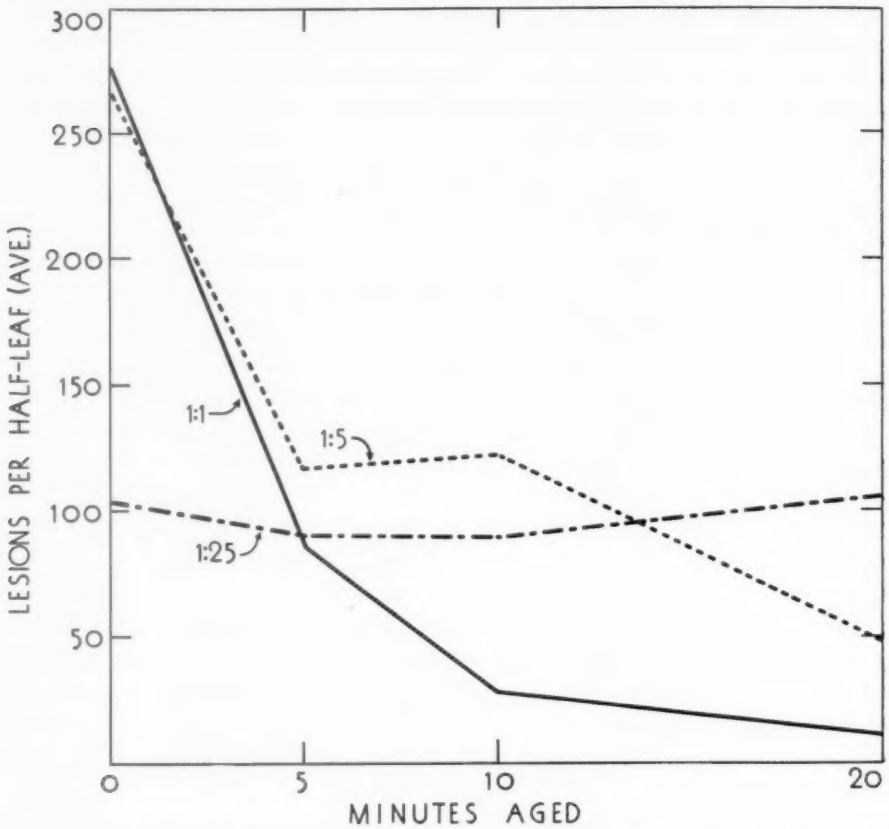

FIG. 1. The effect of the concentration of inoculum on the rate of inactivation of the tobacco streak virus by aging.

vator in the crushed tissue, in spite of attempts to make inoculations as rapidly as possible. Apparently in the more dilute inocula the concentration of inactivator formed was too low to have a detectable effect.

The primary cause of the rapid "aging" of the virus in extracts was the formation of apparently the same inactivator as appeared when healthy tissue was crushed and aged. In an attempt to determine whether both types of inactivation were due to the same cause, the relation of the age

of the infected leaf to the rate of aging of the virus was determined. In one trial, tissue from an inoculated upper tobacco leaf, diluted with 4 parts of buffer, gave an average of 246 lesions per half-leaf of guar when inoculated immediately, and an average of only 5.5 lesions after standing 20 minutes. The same concentration of inoculum from an inoculated lower leaf of the same plant produced an average of 272 lesions when inoculated immediately, and an average of 166 lesions after standing 20 minutes. The results of other trials were similar, except in a few instances, when inoculum from lower leaves lost infectivity nearly as rapidly as that from upper leaves.

Determinations were then made of the inactivating effect of fresh and aged healthy tissue from upper and lower tobacco leaves comparable to the infected leaves used in the preceding trials. A 1:25 preparation of virus was treated either by grinding with a total of 20 per cent healthy tissue and inoculating immediately, or by grinding the healthy tissue first, allowing it to stand for 20 minutes, and then grinding the infected tissue with this pulp and inoculating. In one trial the unaged healthy tissue from an upper leaf inactivated 32 per cent of the virus, as compared to the control containing no healthy tissue. The aged extract from the same leaf inactivated 98.5 per cent of the virus. The unaged extract from the lower leaf caused no inactivation, while after aging 20 minutes it inactivated 62 per cent of the virus. Other trials gave similar results. The increased rate of aging of the virus in extracts of younger leaves was correlated with the increased rate of formation of inactivator in extracts of younger, healthy leaves of the same size and age.

Since the inactivator appeared in extracts after the tissue had been crushed and exposed to air, and since it appeared to approximately the same extent in extracts of both healthy and diseased leaves, it is not likely that it is related to the phenomenon of acquired tolerance. It was further found that rapid aging of the tobacco streak virus occurs in extracts of infected *Nicotiana glutinosa* and *N. rustica*, plants which do not react to the virus in the same manner as *N. tabacum*.

Relation of Oxidation to the Formation of Inactivator

The evidence suggested that the inactivating substance appearing in extracts of tobacco leaves was the result of an oxidation. To test this possibility, apparatus was devised by which tissue could be crushed in a perforated test tube which was sealed within a small flask by rubber connections. Before crushing the tissue, the oxygen in the flask was exhausted by an alkaline solution of pyrogallol. In every trial, crushed tissue held for 20 minutes in an oxygen-free atmosphere lost little or no infectivity. In control experiments, omitting only the pyrogallol, extracts lost 96 to 99 per cent of their infectivity within 20 minutes.

The results are similar to results described by Bald and Samuel (2)

and Best and Samuel (4) with the tomato spotted wilt virus. These investigators, and later Best (3), also showed that the presence of reducing agents in the inoculum had a preservative effect on the virus of tomato spotted wilt. Similarly, Ainsworth and Ogilvie (1) showed that lettuce mosaic was more infectious and could be preserved longer when sodium sulphite was added to the inoculum. Because the rate of inactivation of the tobacco streak virus was not the same in all preparations, some method of preventing the inactivation was necessary in making quantitative comparisons. Several reducing agents were tested for this purpose. One part

TABLE 2.—*The preservative effect of certain reducing agents on the tobacco streak virus in preparations of 1 part of infected tissue ground in 4 parts of phosphate buffer (pH 7.0) containing the reducing agent*

Reducing agent	Trial	Average lesions per half-leaf of guar produced by preparations:			
		Containing reducing agent		Without reducing agent	
		Unaged	*Aged 20 min.*	*Unaged*	*Aged 20 min.*
Cysteine hydrochloride (0.01 molar)	I	364	395	119	24
	II	73	69	131	2
	III	211	172	199	2
Glutathione (0.01 molar)	I	354	309	77	17
	II	136	16	139	2
	III	138	155	208	3
Sodium thioglycollate (0.01 molar)	I	265	159	58	9
	II	64	59	55	1
	III	187	193	204	5
Potassium cyanide (0.01 molar)	I	251	19	141	8
	II	84	5	130	5
	III	336	28	296	22
Sodium sulphite (0.01 molar)	I	465	371	148	26
	II	146	89	159	9
	III	127	144	172	14
Iron filings (40 mg. per ml.)	I	299	149	87	14
	II	153	108	123	3
	III	278	92	289	11

of infected tobacco leaf tissue was ground in 4 parts of 0.01 molar solutions (or 40 mg. per ml. of iron filings) of the reducing agent in pH 7 phosphate buffer. Inoculations with this preparation were made immediately, and after 20 minutes, to half-leaves of guar. As controls, similar preparations containing no added reducing agent were inoculated immediately, and after 20 minutes, to the opposite half-leaves of the test plants. The results of 3 trials with 6 reducing agents are presented in table 2.

In the presence of 0.01 M concentrations of cysteine hydrochloride, glutathione, sodium thioglycollate, sodium sulphite, and 40 mg. per ml. of iron filings, the infectivity of the preparations remained nearly constant for 20 minutes, or decreased less than did the infectivity of control prepa-

rations. In all trials, inoculum without an added reducing agent gave very few lesions after being aged 20 minutes.

For each of the 3 trials, all the inoculum was taken from a single infected tobacco leaf in an effort to minimize any differences in the tissue to be treated by different reducing agents. In trial I, the presence of each of the 6 substances resulted in a considerably greater number of lesions than was produced by the control inoculum, even though the control was inoculated within 1 minute after grinding the tissue. This effect was produced by potassium cyanide, although it had little or no preservative effect on the virus. Either a reactivation of virus occurred in the treated extracts, or a very rapid inactivation of virus occurred in the control extracts. Further trials showed that, when cysteine hydrochloride was added to inoculum which had been ground and aged 20 minutes, there was no increase in infectivity as compared to the aged control. It was concluded that once the virus was inactivated it could not be reactivated by a reducing agent. Apparently, also, an appreciable inactivation of virus may occur within a very short time in some extracts.

Virus Concentration in Relation to Acquired Tolerance

After it was found that the rapid inactivation of the tobacco streak virus in extracts could be prevented by including a reducing agent in the inoculum, another series of comparisons was made of virus concentration in necrotic and tolerant leaves. The experimental procedure was the same as had been used previously, except that the buffer in which the disks were ground contained 0.01 molar cysteine hydrochloride, freshly dissolved. The infectivity of 3 tissue concentrations from necrotic leaves was com-

TABLE 3.—*The relative concentration of tobacco streak virus in necrotic and tolerant leaves of Havana 38 tobacco. All inoculum contained 0.01 molar cysteine hydrochloride*

Tissue concentration	Source of inoculum	Total lesions on 3 half-leaves of guar in each of 6 trials					
		I	II	III	IV	V	VI
1: 5	Necrotic leaves	554	313	491	313	370	484
	Tolerant leaves	633	569	832	257	418	867
1: 25	Necrotic leaves	555	88	340	138	233	316
	Tolerant leaves	641	385	689	136	288	606
1: 125	Necrotic leaves	108	5	5	10	15	33
	Tolerant leaves	290	68	180	37	93	196
Ratio of infectivities, Necrotic/tolerant[a]	Log ratio and semi-confidence interval (t 0.05)	−0.23 ±0.20	−0.52 ±0.22	−0.17 ±0.22	−0.07 ±0.07	−0.34 ±0.19	−0.23 ±0.20
	Ratio	0.58	0.30	0.67	0.85	0.46	0.58

[a] The method of calculation was that described by Price and Spencer (12).

pared on opposite half-leaves of guar with the infectivity of the same 3 tissue concentrations from tolerant leaves. The results are presented in table 3.

In comparing the infectivities of the tissues, the ratio of the infectivity of necrotic tissue to the infectivity of tolerant tissue was determined. The log of this ratio and its semi-confidence interval (standard error multiplied by t 0.05) were calculated by the method described by Price and Spencer (12).[1] In all cases the ratio (antilog) was less than 1. The tolerant tissue evidently contained more active virus per unit of area than did the necrotic tissue. This may have been due partly to the fact that a small proportion of the total area of tissue from necrotic leaves was composed of dead cells, which presumably contained no virus. The tissue from tolerant leaves, on the other hand, showed no observable necrotic areas.

TABLE 4.—*The relative concentration of tobacco ringspot virus in necrotic and tolerant leaves of Havana 38 tobacco*

Tissue concentration	Source of inoculum	Total lesions on 2 half-leaves of cowpea in each of 7 trials						
		I	II	III	IV	V	VI	VII
1:250	Necrotic leaves	302	137	294	406	378	409	274
	Tolerant leaves	303	163	453	425	364	411	307
1:1250	Necrotic leaves	68	20	63	94	147	111	132
	Tolerant leaves	57	30	100	153	138	115	161
1:6250	Necrotic leaves	6	2	3	11	16	11	4
	Tolerant leaves	4	3	16	18	16	10	7
Ratio of infectivities, Necrotic/tolerant[a]	Log ratio and semi-confidence interval (t 0.05)	.039 ± .017	−.054 ± .024	−.209 ± .143	−.184 ± .049	.037 ± .133	.007 ± .016	−.044 ± .028
	Ratio	1.09	0.88	0.62	0.65	1.09	1.00	0.90

[a] The method of calculation was that described by Price and Spencer (12).

The results do not show a lower concentration of tobacco streak virus in tolerant leaves, and they differ from those of Price (11) on the tobacco ringspot virus in this respect. His comparisons, also made between equal areas of different leaves, showed that the concentration of tobacco ringspot virus was considerably lower in tolerant leaves than in necrotic leaves. Since his determinations probably were made under somewhat different conditions, they were repeated, using ringspot-infected Havana 38 tobacco plants grown under the same conditions as the streak-infected plants. Tissue samples were taken with a sterile cork borer, and the inocula prepared as with tobacco streak. Since it appeared possible that the presence of a reducing agent might increase infectivity, several trials were run to determine the effect of 0.01 molar cysteine hydrochloride on the tobacco

[1] The writer wishes to thank Professor James H. Torrie of the Department of Agronomy, University of Wisconsin, for advice on the application of this method to the results.

ringspot virus. No significant difference in infectivity was found between extracts with and those without this reducing agent. There was no detectable loss of infectivity of tobacco ringspot virus extracts, without the reducing agent, within 20 minutes. Consequently, inocula were prepared with only pH 7 phosphate buffer. Three tissue concentrations, 1:250, 1:1250, and 1:6250, from necrotic and tolerant leaves were applied to opposite halves of primary cowpea leaves. Three cowpea plants, providing

TABLE 5.—*The relative concentrations of tobacco streak and tobacco ringspot viruses in necrotic and tolerant leaves of Xanthia tobacco. The tobacco streak inoculum contained 0.01 molar cysteine hydrochloride*

Tissue concentration	Source of inoculum	Total lesions on 3 half-leaves of guar in each of 5 trials				
Tobacco streak virus		I	II	III	IV	V
1:5	Necrotic leaves	494	300	320	99	109
	Tolerant leaves	507	297	608	378	120
1:25	Necrotic leaves	236	230	149	18	11
	Tolerant leaves	255	237	374	75	25
1:125	Necrotic leaves	54	31	3	0	0
	Tolerant leaves	64	44	16	6	2
Ratio of infectivities, Necrotic/tolerant[a]	Log ratio and semi-confidence interval (t 0.05)	−0.034	−0.057	−0.221	−0.291	−0.100
		±0.02	±0.046	±0.089	±0.106	±0.041
	Ratio	0.92	0.87	0.60	0.51	0.79
Tobacco ringspot virus		Total lesions on 2 half-leaves of cowpea				
1:250	Necrotic leaves	329	430	267	336	294
	Tolerant leaves	130	220	131	157	48
1:1250	Necrotic leaves	75	65	55	103	50
	Tolerant leaves	9	19	12	34	5
1:6250	Necrotic leaves	7	5	4	12	3
	Tolerant leaves	0	2	0	3	0
Ratio of infectivities, Necrotic/tolerant[a]	Log ratio and semi confidence interval (t 0.05)	0.365	0.191	0.208	0.323	0.379
		±0.076	±0.063	±0.060	±0.054	±0.101
	Ratio	2.32	1.55	1.61	2.10	2.39

[a] The method of calculation was that described by Price and Spencer (12).

a total of 12 half-leaves, were used in each trial. The results of 7 trials are presented in table 4.

The infectivity of tobacco ringspot virus extracts from tolerant leaves was at least as great as that from necrotic leaves. Similar results were obtained in a series of comparisons using guar as an assay plant. The results were consistent with those on the tobacco streak virus in indicating that tolerant leaves contained as much or more virus than necrotic leaves.

A factor which might have contributed to the difference between the results in this investigation and those of Price (11) was the use of differ-

ent types of tobacco. Since his studies were made on Turkish tobacco, additional trials were made with each virus, using infected plants of the Turkish variety Xanthia. The results are presented in table 5.

In contrast to the results with Havana 38 tobacco, the concentration of the tobacco ringspot virus appeared considerably lower in the tolerant leaves of the Turkish tobacco, as was reported by Price (11). With the tobacco streak virus, on the other hand, the concentration of virus in the tolerant leaves was slightly greater than in the necrotic leaves.

From the foregoing results it was concluded that a low virus content is not correlated with the absence of necrosis of the uppermost leaves of plants infected with the two viruses. Although the new upper leaves display only slight symptoms, apparently multiplication of the viruses in them may be unrestricted.

Virulence of the Virus in Tolerant Leaves

Since the symptomless condition of the upper leaves of streak-infected tobacco could not be explained by any restriction imposed by the plant upon multiplication of the virus, another possibility was considered. If a mild strain of the virus should appear in infected plants and move rapidly into the growing tip, it might prevent the invasion or production of symptoms by the more severe strain of virus. To test this possibility, material from tolerant leaves of streak-infected tobacco was inoculated to the primary leaves of Great Northern bean. The local lesions which developed were removed separately with a sterile cork borer and inoculated to healthy tobacco. A total of 148 single lesions produced the same necrotic symptoms characteristic of the original disease. In these trials, three strains of tobacco streak were used, distinguishable from each other by the type and severity of the necrotic symptoms. The single-lesion isolates from tolerant leaves in every case were identical to the strain of virus originally infecting the plant. It was concluded that no change in virulence of the virus had occurred in the tolerant leaves.

DISCUSSION

The investigation was undertaken with the hope of adding information to the subject of acquired tolerance to virus diseases in plants. The tobacco streak virus seemed especially suitable for such a study. Comparisons also were made with the tobacco ringspot virus, particularly with regard to relative concentration of virus in different tissues. The sequence of symptoms of plants infected with each of the viruses is similar. With the strain of tobacco streak virus used, the "recovery" of infected plants is more rapid and more complete than with the tobacco ringspot virus. The data, however, showed no fundamental differences in the behavior of each virus within the host. The properties of the two viruses, on the other hand, are not similar so far as is known. It seems possible that a

closer comparison might reveal certain properties which are similar and which might have a bearing on the similar reaction of plants infected with these viruses.

The presence of tobacco ringspot virus in relatively low concentration in tolerant leaves of Turkish tobacco, and in high concentration in tolerant leaves of Havana tobacco, could not be correlated with any visible difference in the reaction of the plants to the virus. It does not seem probable that the low concentration of virus is directly related to the absence of symptoms in one variety and not in the other. It seems more likely that the low concentration in tolerant leaves of Turkish tobacco is not causally related to the type of symptom.

The data obtained show that the tobacco streak virus is present in the symptomless leaves of infected plants in undiminished concentration and virulence. What appears to be a change in the reaction of the plant to the virus occurs between the initial infection and the development of new leaves following the systemic movement of the virus. This change, however, may be due to a change in the location or distribution of the virus. Price (11) has suggested that embryonic cells of the stem tip become adapted to the presence of the tobacco ringspot virus, and are able to develop nearly normally in spite of the virus. Valleau (15) has postulated a more or less complete invasion of the embryonic tissue by the virus, and that the subsequent formation of new leaves uniformly invaded by the virus does not result in the line and ring type of symptom. In the present investigation some trials were made which, while not conclusive, indicated that the embryonic tissue in the stem tips of infected plants contained as much virus per unit weight as any other part of the plant. Since the necrotic lines characteristic of tobacco streak seem to develop near the advancing margin of the virus as it invades a leaf, it is possible that young leaves formed with a uniform distribution of virus within them would react differently.

McKinney and Clayton (9) have interpreted the development of the "chronic phase" of ringspot as being due to a high degree of resistance to necrosis in the young leaves at the time they are invaded. In streak-infected tobacco, also, the youngest bud leaves do not become necrotic when systemic symptoms develop on older leaves. Almost invariably the youngest leaf to show symptoms will be necrotic only at its tip, the most nearly mature portion. Whether the bud leaves continue to grow without becoming necrotic because they retain this resistance, or because they are subject to a different invasion pattern of virus, or for some other reason, cannot be answered on the basis of the present data.

The terms which have been used to describe the development of non-necrotic leaves on ringspot-infected plants have been based on different interpretations of experimental results. Price (10) applied the term "acquired immunity" to plants infected with the tobacco ringspot virus be-

cause they produced symptomless leaves which did not become necrotic when reinoculated. Since such leaves are immune from a set of symptoms and not from the virus, objections have been raised to the application of the word "immunity." Similar objections have been raised to the use of the term "recovery." Valleau (15) and McKinney and Clayton (9) would prefer to use the term "chronic disease."

The term acquired immunity has been applied also to protective inoculation, including cases where infection with the protecting strain of virus does not lead to the appearance of symptomless leaves. This protection or immunization appears to depend chiefly upon complete invasion by the protecting virus. That symptomless leaves of ringspot or streak-infected plants are protected against reinoculation is apparent. That this protection is the cause of the development of symptomless leaves has not been demonstrated. It is likely that immunity to reinoculation is caused by factors different from those which lead to the development of symptomless leaves. It would seem better, therefore, to use separate terms which would express more precisely the intended meaning.

In this paper the term "acquired tolerance," as suggested by Smith (13), has been used because it most adequately expresses the actual condition of the infected plant. The term "chronic disease" might also be used, but it was felt that this did not sufficiently emphasize the symptomless condition which is so characteristic of the tobacco streak disease.

The formation of an inactivator of the tobacco streak virus by oxidation of some component in extracts of healthy or diseased tobacco presents some interesting possibilities for further study. Susceptibility to the same type of inactivator as affects the tomato spotted wilt virus suggests that there may be other viruses equally, or more, susceptible to the same type of inactivator. The addition of reducing agents to inoculum might permit mechanical transmission in some cases where otherwise it is difficult or impossible.

SUMMARY

When attempts were made to compare the concentration of tobacco streak virus in necrotic leaves with the concentration in symptomless new leaves, it was found that infectivity increased with increasing dilution. This reversal of the normal dilution curve was found to be due to the formation of an inactivator when extracts of diseased or healthy tobacco leaves were exposed to air. Because the inactivator appeared only after crushing the tissue, and because healthy and diseased leaves were equally good sources, it was concluded to be unrelated to the phenomenon of acquired tolerance.

By adding reducing agents (cysteine hydrochloride, glutathione, sodium thioglycollate, sodium sulphite, iron filings) to the inoculum at the time the tissue was ground, rapid inactivation of the virus was prevented. Untreated inoculum lost 75 to 99 per cent of its infectivity within 20 minutes.

When infected tissue for assay was ground in the presence of a reducing agent, the concentration of tobacco streak virus was found to be as high, or higher, in tolerant leaves as in necrotic leaves of both Havana 38 and Xanthia varieties of tobacco. The concentration of the tobacco ringspot virus in infected Havana 38 tobacco was approximately the same in tolerant and necrotic leaves. In Xanthia tobacco, there was a higher concentration of this virus in necrotic leaves than in tolerant leaves.

Single lesion isolations of tobacco streak virus were made from bean leaves inoculated with tolerant tobacco leaves. The single lesion strains from tolerant leaves were as virulent as those from necrotic leaves.

It was concluded that the production of new symptomless leaves on plants infected with the tobacco streak virus is not due to any reduction in the amount of virus or in the virulence of the virus present.

DIVISION OF TOBACCO, MEDICINAL AND SPECIAL CROPS,
U. S. DEPARTMENT OF AGRICULTURE
AND
WISCONSIN AGRICULTURAL EXPERIMENT STATION,
MADISON, WISCONSIN

LITERATURE CITED

1. AINSWORTH, G. C., and L. OGILVIE. Lettuce mosaic. Ann. Appl. Biol. **26**: 279–297. 1939.
2. BALD, J. G., and GEOFFREY SAMUEL. Some factors affecting the inactivation rate of the virus of tomato spotted wilt. Ann. Appl. Biol. **21**: 179–190. 1934.
3. BEST, RUPERT J. The preservative effect of some reducing systems on the virus of tomato spotted wilt. Austr. Jour. Exp. Biol. and Med. Sci. **17**: 1–17. 1939.
4. ———, and GEOFFREY SAMUEL. The effect of various chemical treatments on the activity of the viruses of tomato spotted wilt and tobacco mosaic. Ann. Appl. Biol. **23**: 759–780. 1936.
5. DIACHUN, STEPHEN. Inhibition of tobacco streak virus by juice from healthy plants. (Abstr.) Phytopath. **38**: 7. 1948.
6. FULTON, ROBERT W. Hosts of the tobacco streak virus. Phytopath. **38**: 421–428. 1948.
7. JOHNSON, JAMES. An acquired partial immunity to the tobacco streak disease. Trans. Wis. Acad. Sci. Arts and Letters **30**: 27–34. 1937.
8. KUNTZ, J. E., and J. C. WALKER. Virus inhibition by extracts of spinach. Phytopath. **37**: 561–579. 1947.
9. MC KINNEY, H. H., and E. E. CLAYTON. Acute and chronic symptoms in the tobacco ring-spot disease. Phytopath. **34**: 60–76. 1944.
10. PRICE, W. C. Acquired immunity to ring-spot in Nicotiana. Contrib. Boyce Thomp. Inst. **4**: 359–403. 1932.
11. ———. Virus concentration in relation to acquired immunity from tobacco ring spot. Phytopath. **26**: 503–529. 1936.
12. ———, and ERNEST L. SPENCER. Accuracy of the local lesion method for measuring virus activity. III. The standard deviation of the log-ratio of potencies as a measure of the accuracy of measurement. Amer. Jour. Bot. **30**: 720–735. 1943.
13. SMITH, K. M. The present status of plant virus research. Biol. Rev. **8**: 136–179. 1933.
14. STANLEY, W. M. Isolation of virus from plants recovered from the tobacco ring spot disease. Jour. Biol. Chem. **129**: 429–436. 1939.
15. VALLEAU, W. D. Experimental production of symptoms in so-called recovered ring-spot tobacco plants and its bearing on acquired immunity. Phytopath. **31**: 522–533. 1941.